BEST AVAILABLE IMAGES

Defective images within this document are accurate representation of The original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

PCT WORLD INTELLECTUAL PROPERTY ORGANIZATION
International Bureau

INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 6 :
A61K 38/55, 38/10, 38/03, C12N 9/50

A1

(11) International Publication Number: **WO 98/37910**

(43) International Publication Date: 3 September 1998 (03.09.98)

(21) International Application Number: PCT/US98/03642

(22) International Filing Date: 25 February 1998 (25.02.98)

(30) Priority Data:
60/039,129 2 March 1997 (02.03.97) US

(71) Applicant *(for all designated States except US)*: EMORY UNIVERSITY [US/US]; 1380 South Oxford Road, Atlanta, GA 30322 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: NOE, Bryan, D. [US/US]; 1721 Stonecliff Court, Decatur, GA 30033 (US). ROTHENBERG, Mark, E. [US/US]; 944 Cedar Crest Court, Lilburn, GA 30047 (US).

(74) Agents: PRATT, John, S. et al.; Kilpatrick Stockton LLP, Suite 2800, 1100 Peachtree Street, Atlanta, GA 30309-4530 (US).

(81) Designated States: AU, CA, JP, US, European patent (AT, BE, CH, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE).

Published
With international search report.
Before the expiration of the time limit for amending the claims and to be republished in the event of the receipt of amendments.

(54) Title: PEPTIDE INHBITORS OF PROPEPTIDE/PROHORMONE CONVERTASES

(57) Abstract

Peptide inhibitors of propeptide/prohormone convertase (PC) selected from the group consisting of (I) and longer peptides containing said peptides are described. Such peptides, in addition to being effective in the inhibition of PC, are useful in the treatment of tumor cells and in the treatment of endocrine disorders.

RGDVACTKQFDPVVVT (SEQ ID NO: 1),
GEDVMCPMVYDPVLLT (SEQ ID NO: 2),
DTGRVCTREYRPVTVS (SEQ ID NO: 3),
RDGVICNKLYDPVVVT (SEQ ID NO: 4),
ADGVMCTREYAPVVVT (SEQ ID NO: 5),
SEGVMCPMIYDPVLLT (SEQ ID NO: 6),
CALEGSLQKRGIVEQCC (SEQ ID NO: 7),
CALEGSLQKHGIVEQCC (SEQ ID NO: 8),
CRDGVICNKLYDPVVVTC (SEQ ID NO: 9),
CADGVMCTREYAPVVVTC (SEQ ID NO: 10),
CDTGRVCTREYRPVTVSC (SEQ ID NO: 11),
CRGDVACTKQFDPVVVTC (SEQ ID NO: 13), (I)
CGEDVMCPMVYDPVLLTC (SEQ ID NO: 14),
CSEGVMCPMIYDPVLLTC (SEQ ID NO: 15),
RDGVICNKNYDPVVVT (SEQ ID NO: 16),
DDAVMCTREYAPVVVT (SEQ ID NO: 17),
DQDRACIKIYDPLVVT (SEQ ID NO: 18),
DAGVMCTREYAPVVVT (SEQ ID NO: 19),
RDGVMCTKQYDPVVVT (SEQ ID NO: 20),
EPGRMCTKEWRPITVT (SEQ ID NO: 21),
RDDVWCNKLYDPVVVT (SEQ ID NO: 22),
EDSVMCTREYAPVVVT (SEQ ID NO: 23),
TGDVMCTKQYDVVVT (SEQ ID NO: 24),

FOR THE PURPOSES OF INFORMATION ONLY

Codes used to identify States party to the PCT on the front pages of pamphlets publishing international applications under the PCT.

AL	Albania	**ES**	Spain	**LS**	Lesotho	**SI**	Slovenia
AM	Armenia	**FI**	Finland	**LT**	Lithuania	**SK**	Slovakia
AT	Austria	**FR**	France	**LU**	Luxembourg	**SN**	Senegal
AU	Australia	**GA**	Gabon	**LV**	Latvia	**SZ**	Swaziland
AZ	Azerbaijan	**GB**	United Kingdom	**MC**	Monaco	**TD**	Chad
BA	Bosnia and Herzegovina	**GE**	Georgia	**MD**	Republic of Moldova	**TG**	Togo
BB	Barbados	**GH**	Ghana	**MG**	Madagascar	**TJ**	Tajikistan
BE	Belgium	**GN**	Guinea	**MK**	The former Yugoslav Republic of Macedonia	**TM**	Turkmenistan
BF	Burkina Faso	**GR**	Greece			**TR**	Turkey
BG	Bulgaria	**HU**	Hungary	**ML**	Mali	**TT**	Trinidad and Tobago
BJ	Benin	**IE**	Ireland	**MN**	Mongolia	**UA**	Ukraine
BR	Brazil	**IL**	Israel	**MR**	Mauritania	**UG**	Uganda
BY	Belarus	**IS**	Iceland	**MW**	Malawi	**US**	United States of America
CA	Canada	**IT**	Italy	**MX**	Mexico	**UZ**	Uzbekistan
CF	Central African Republic	**JP**	Japan	**NE**	Niger	**VN**	Viet Nam
CG	Congo	**KE**	Kenya	**NL**	Netherlands	**YU**	Yugoslavia
CH	Switzerland	**KG**	Kyrgyzstan	**NO**	Norway	**ZW**	Zimbabwe
CI	Côte d'Ivoire	**KP**	Democratic People's Republic of Korea	**NZ**	New Zealand		
CM	Cameroon			**PL**	Poland		
CN	China	**KR**	Republic of Korea	**PT**	Portugal		
CU	Cuba	**KZ**	Kazakstan	**RO**	Romania		
CZ	Czech Republic	**LC**	Saint Lucia	**RU**	Russian Federation		
DE	Germany	**LI**	Liechtenstein	**SD**	Sudan		
DK	Denmark	**LK**	Sri Lanka	**SE**	Sweden		
EE	Estonia	**LR**	Liberia	**SG**	Singapore		

PEPTIDE INHIBITORS OF PROPEPTIDE/PROHORMONE CONVERTASES

The U.S. Government has certain rights in the invention, based on grant support from National Institutes of Health No. DK 44986.

BACKGROUND OF THE INVENTION

Field of the Invention

The present invention pertains to peptide inhibitors of propeptide/prohormone convertases, to a method of inhibiting propeptide/prohormone convertases, to a method of treating cancer, endocrine disorders, and viral infections including AIDS, and, more specifically, to a method of treating cancer, endocrine disorders, and viral infections by means of peptide inhibitors of propeptide/prohormone convertases.

Description of Related Art

Regulation of many physiologic processes requires the action of biologically active peptides which are first synthesized as larger inactive precursors. These precursors are subsequently altered by various post-translational modifications. Two examples of bioactive peptide hormones that are enzymatically cleaved from precursors are the pancreatic islet peptides insulin and glucagon. These prohormones require proteolytic cleavage by endoproteases at pairs of basic amino acid residues before the bioactive products are secreted. These endopeptidases were first characterized in the mid 1980's, with the discovery of a yeast endoprotease responsible for processing substrates at basic amino acid pairs. The gene was called Kex2, and its product was found to process two yeast pheromones, pro-α mating factor and pro-killer toxin. Later, several mammalian homologs were identified and characterized. Currently there are more than ten known endoproteases or Prohormone/Propeptide Convertases (PCs). They include the mammalian PC1/3 (also referred to as SPC3 or PC3), PC2, the PACE (Paired Amino acid Converting Enzyme) family of PCs, furin,

PC4, PC5/6 (PC6A) PC6B, and PC7. PC1/3 and PC2 are expressed predominantly within endocrine and neuroendocrine cells and tissues, while furin, PACE4 and PC6 isoforms are expressed ubiquitously. PC6A has been localized only within a subset of endocrine and non-endocrine cells (for example, pancreatic islets and gut endocrine cells), while PC4 is expressed primarily within testicular germ cells. See P.A. Bresnahan et al. (1990) *J. Cell Biol.* 111:2851-2859; J. Korner et al. (1991) *Proc. Nat. Acad. Sci. USA* 88:6834-6838; R.B. Mackin et al. (1987) *J. Biol. Chem.* 262:6453-6456; and K. Nakayama et al. (1991) *J. Biochem.* (Tokyo) 109:803-806. As most propeptides or prohormones exhibit minimal biological activity, the PCs play an essential role in generating bioactive product peptides from these precursors.

Previous studies have indicated that some of the PCs, particularly PCs localized to the regulated secretory pathway in neuroendocrine cells and PCs ectopically expressed in tumor cells, are involved in exacerbating the pathophysiology of many different types of cancer. Prime candidates are PC1/3, PC2, and PC6A. See J. Lusson et al. (1993) *Proc. Natl. Acad. Sci. USA* 90:6691-6695 and T. Nakagawa et al. (1993) *J. Biochem.* 113:132-135. There are two separate ways in which PCs are involved. First, tumors of neuroendocrine cell origin often hypersecrete peptide hormones. See J.F Rehfield et al. (1995) *Front. Gastrointest. Res.* 23:84-98. Examples include insulinomas, glucagonomas, somatostatinomas, gastrinomas (Zollinger-Ellison Syndrome), tumors of the anterior pituitary (ACTH or growth hormone hypersecretion), among others. In every one of these cases in which bioactive peptides are released from tumor cells, afflicted individuals suffer severe physiologic consequences as a result of peptide hormone hypersecretion. The PCs play an essential role in mediating the metabolic consequences of peptide hormone hypersecretion in that their activity is responsible for cleaving the biologically inactive precursors of the bioactive peptides.

PCs can also augment cancer pathobiology by promoting tumor growth and proliferation. This role is again mediated by the precursor processing activity of the PCs. However, in this context the substrates are precursors to growth factors such

as epidermal growth factor (EGF), the family of insulin-like growth factors (IGFs), nerve growth factors such as NGF-β, neurotrophin, platelet derived growth factors (PDGFs), or transforming growth factor beta (TGF-β). See M. Mbikay et al. (1993) *Ann. NY Acad. Sci.* 680:13-19. All of these growth factors are known to be synthesized by precursors subjected to post-translational processing at single or multiple basic amino acid sites for activation. It is also known that expression of growth factors and their receptors is elevated in numerous types of transformed cells, and it has been suggested that cancer cells produce their own hormone-like growth factors which bind to receptors on their cells of origin, stimulating growth in an autocrine fashion. Moreover, transfection of normal cells with vectors coding for growth factors can result in transformation of the transfected cells. It has been demonstrated that the tumorigenicity of such transformed cells can be reversed by administration of growth factor antagonists, or by preventing growth factor expression or growth factor receptor expression with antisense approaches. See S. Mahmoud et al. (1991) *Cancer Res.* 51:1798-1802; T.W. Moody et al. (1995) *Life Sci.* 56:521-529; J. Trojan et al. (1992) *Proc. Natl. Acad. Sci. USA* 89:4874-4878; and S. Neuenschwander et al. *Endocrinology* 136:4298-4303 (1995).

Previous studies have also indicated that some PCs play a role in establishing viral infections by processing the precursor envelope glycoproteins of viral pathogens, including HIV. See J. McCune (1988) *Cell* 53: 55-67. Infectivity of HIV depends upon the interaction between gp120 displayed on the virion surface and CD4+ receptors on the surface of the target cell. See S. Bour et al. (1995) *Microbiol. Rev.* 59:63-93. Surface gp120 can also promote syncytium formation between infected and juxtaposed CD4+ cells, further compromising the infected patient's immune system. See J. Levy (1993) *Microbiol. Rev.* 57: 183:289. It has been shown that gp120, and the non-covalently associated gp41, are cleavage products of gp160, a precursor protein initially synthesized in the host cell and sorted into the constitutive secretory pathway. See R. Wukket et al. (1988) *Proc. Natl. Acad. Sci USA* 85:9580-9584 and M. Kowalski et al. (1987) *Science* 237: 1351-1355.

The gp160 cleavage site, REKR ↓ AV, fits the furin cleavage site consensus sequence REKR. F. Veronese et al. (1985) *Science* 229: 1402-1405 and Hatsuzawa et al. (1992) *J. Biochem. (Tokyo)* 111: 296-301. This site is highly conserved in both HIV-1 and HIV-2, as well as in other viral coat glycoproteins. See R. Brasseur et al. *Biochim. Biophys. Acta* 1029: 267-273; M. Vey et al. (1992) *Virology* 188: 408-413; E. Fenouillet et al. (1992) *Virology* 187: 825-828; and S. Zarkik (1997) *FEBS Lett.* 406: 205-210. Experimental evidence to date demonstrates that processing of gp160 occurs in the trans Golgi network and is a calcium dependent process. See M. Moulard (1994) *FEBS Lett.* 338: 281-284. Collectively, these observations suggest that furin or other proteases of the PC family are likely responsible for the host cell mediated cleavage of gp160 to gp120 and gp41.

It has been shown that furin can mediate cleavage of gp160. See S. Hallenberger et al. (1992) *Nature* 360: 358-361 and E. Decroly et al.(1997) *FEBS Lett.* 405: 68-72. However, the observation that gp160 is also cleaved in furin-deficient cells suggests that proteases other than furin may be responsible for gp160 processing in some cell types. See M. Inocencio (1997) *J. Biol. Chem.* 272: 1344-1348. Experimental evidence from CD4+ cells suggests that furin, PC6 and/or PC7 are primarily responsible for host cell- mediated cleavage of gp160 in HIV target cells. See S. Zarkik et al. (1997) *FEBS Lett.* 406: 205-210 and E. Decroly et al. (1996) *J. Biol. Chem.* 271: 30442-30450.

The effects of synthetic inhibitors on PC activity have been investigated. See H. Angliker et al. (1993) *Biochem. J.* 293:75-81; W. Garten et al. (1994) *Biochimie* 76:217-225; F. Jean et al. (1995) *Biochem. J.* 307:689-695; and A. Basak et al.(1994) *Int. J. Pept. Protein Res.* 44:253-261. However, the inhibitors employed in these studies were either classic chemical proteinase inhibitors or synthetic peptides with bulky blocked end groups and/or chemical side chains. Thus, there remained a need for propeptide/prohormone convertase inhibitors that were either small peptides or full length proteins. Such inhibitors are expressed in intact cells and targeted to the regulated secretory pathway. In this way, a constantly renewable supply of inhibitor is

supplied in the trans Golgi network (to inhibit furin) and in secretory granules (to inhibit PCs) by constitutive or regulated expression.

These inhibitors is expressed and targeted into the regulated secretory pathway of mammalian neuroendocrine cells where they are suitable as reagents in reducing malignant transformation and tumorigenesis in cancer cells. Inhibition of peptide prohormone conversion also reduces the serious physiologic consequences of tumors that produce and release bioactive peptides such as those derived from insulinomas, gastrinomas, or lung cancer cells which often hypersecrete a variety of hormonally active peptides. Inhibition of prohormone conversion would reduce or block the release of the bioactive product peptides from such tumors. In addition, inhibition of peptide prohormone conversion could inhibit neoplasia by blocking PC-mediated processing of growth factors which are produced in many types of tumor cells. These endogenously produced growth factors operate in an autocrine fashion to stimulate malignant transformation and tumorigenesis. If maturation of these growth factors are reduced or prevented, growth and proliferation of tumor cells would be inhibited. However, such results had not been obtained in the prior art.

Peptide inhibitors are suitable in the treatment of viral infections, including HIV infections. PC inhibitors are expressed and targeted into the regulated secretory pathway of HIV target cells, where they inhibit processing of gp160, block formation of gp120, thereby diminishing the infectivity of newly synthesized virions. Formation of syncytia also decreases, contributing to a stabilization of the infected patient's immune system. Thus, if processing of gp160 are reduced or prevented, HIV infection is inhibited. Such results have not been obtained in the prior art.

SUMMARY OF THE INVENTION

Accordingly, an object of the present invention is to provide a novel peptide inhibitor of propeptide/prohormone convertase.

It is another object of the present invention to provide a method of suppressing propeptide/prohormone convertase activity utilizing a peptide inhibitor.

It is another object of the present invention to provide a method of treating a tumor cell utilizing a peptide inhibitor of propeptide/prohormone convertase.

It is another object of the present invention to provide a method of treating a hormone-secreting tumor utilizing a peptide inhibitor of propeptide/prohormone convertase.

It is another object of the present invention to provide a method of treating a cell infected with a viral pathogen utilizing a peptide inhibitor of propeptide/prohormone convertase.

It is another object of the present invention to provide a method of treating a cell infected with HIV utilizing a peptide inhibitor of propeptide/prohormone convertase.

It is still another object of the present invention to provide a method of inhibiting overproduction of endocrine or neuroendrocrine hormones which results in pathophysiology.

It is still another object of the present invention to provide a kit for inhibiting propeptide/prohormone convertase.

The above objects, among others, have been achieved by means of a peptide comprising at least one peptide selected from the group consisting of the peptides

RGDVACTKQFDPVVVT (SEQ ID NO: 1),
GEDVMCPMVYDPVLLT (SEQ ID NO: 2),
DTGRVCTREYRPVTVS (SEQ ID NO: 3),
RDGVICNKLYDPVVVT (SEQ ID NO: 4),
ADGVMCTREYAPVVVT (SEQ ID NO: 5),
SEGVMCPMIYDPVLLT (SEQ ID NO: 6),
CALEGSLQKRGIVEQCC (SEQ ID NO: 7),
CALEGSLQKHGIVEQCC (SEQ ID NO: 8),
CRDGVICNKLYDPVVVTC (SEQ ID NO: 9),
CADGVMCTREYAPVVVTC (SEQ ID NO: 10),

CDTGRVCTREYRPVTVSC (SEQ ID NO: 11),
CRGDVACTKQFDPVVVTC (SEQ ID NO: 13),
CGEDVMCPMVYDPVLLTC (SEQ ID NO: 14),
CSEGVMCPMIYDPVLLTC (SEQ ID NO: 15),
RDGVICNKNYDPVVVT (SEQ ID NO: 16),
DDAVMCTREYAPVVVT (SEQ ID NO: 17),
DQDRACIKIYDPLVVT (SEQ ID NO: 18),
DAGVMCTREYAPVVVT (SEQ ID NO: 19),
RDGVMCTKQYDPVVVT (SEQ ID NO: 20),
EPGRMCTKEWRPITVT (SEQ ID NO: 21),
RDDVWCNKLYDPVVVT (SEQ ID NO: 22),
EDSVMCTREYAPVVVT (SEQ ID NO: 23),
TGDVMCTKQYDVVVT (SEQ ID NO: 24),

and longer peptides containing said peptides.

The above objects, among others, have also been achieved by means of a method of suppressing propeptide/prohormone convertase activity comprising the steps of treating a propeptide/prohormone convertase with a peptide selected from the above-described group and monitoring the degree of inhibition of propeptide/prohormone convertase.

Further, the above objects, among others, have been achieved by means of a method of treating a tumor cell comprising the steps of treating one or more tumor cells with a peptide selected from the above-described group and monitoring the degree of inhibition of propeptide/prohormone convertase.

The above objects, among others, have also been achieved by means of a method of treating a viral infection comprising the steps of treating one or more cells infected with a viral pathogen with at least one peptide selected from the above-described group and monitoring the degree of inhibition of propeptide/prohormone convertase.

Further, the above objects, among others, have been achieved by means of a kit for inhibiting propeptide/prohormone convertase comprising at least one peptide selected from the above-described group.

BRIEF DESCRIPTION OF THE DRAWINGS

A more complete appreciation of the invention and many of the attendant advantages thereof will be readily obtained as the same become better understood by reference to the following detailed description when considered in connection with the accompanying drawings, wherein:

Figure 1 depicts several representative members of the furin/PC family along with their tissue distribution;

Figures 2A and 2B represent the effect of the inhibitor Z-Thr-(4-Amph-Gly)$P(oph)_2$ on the processing of glucagon;

Figure 3 illustrates the effects of natural and Streptomyces inhibitor peptides on the ability of PC 1/3 to cleave a fluorogenic substrate;

Figures 4A, 4B, 4C, and 4D depict the effects of Streptomyces inhibitors on PC2 at two concentrations;

Figures 4E, 4F, and 4G represent the comparison of different concentrations of Streptomyces inhibitors;

Figure 5 illustrates the comparison of the effects of full-length STI-2 and 16-amino-acid-long peptides (based on sequences of PSN and SSI) on recombinant PC1/3 cleavage of a fluorogenic substrate;

Figures 6A, 6B, 6C, and 6D depict the effect of proinsulin C-A (KR) and proinsulin C-A (KH) on the ability of PC 1/3 and PC2 to cleave a fluorogenic substrate;

Figures 7A, 7B, 7C, 7D and 7E illustrate the effects of the STI-2 peptide and the modified STI-2(KN) peptide on PC1/3 and PC2 activity;

Figures 8A, 8B, 8C, 8D, 8E, and 8F represent the effects of the STI-2 peptide on PC1/3- or PC2-mediated proglucagon conversion;

Figures 9A, 9B, 9C, 9D, 9E, and 9F illustrate the effects of STI-2(KN) on PC1/3-mediated proglucagon conversion;

Figures 10A, 10B, 10C, 10D, 10E, and 10F illustrate the effects of mutated forms (N72K and N72R) of full-length STI-2 SSI-like inhibitors on PC1/3 and PC2 activity.

Figures 11A, 11B, 11C, and 11D illustrate the effects on full length STI-2 [N72R] on furin or PC6A mediated cleavage.

Figure 12 depicts a schematic of the "carrier" preprosomatostatin and the peptide products of the chimeric cDNA constructs coding for amino acid sequences representing PC inhibitors.

DETAILED DESCRIPTION OF THE INVENTION

The present invention relates to a peptide inhibitor of propeptide/prohormone convertase selected from the group consisting of

CTRXYXPVVVT (SEQ ID NO: 43),
CTKXYXPVVVT (SEQ ID NO: 44),
CNRXYXPVVVT (SEQ ID NO: 45),
CNKXYXPVVVT (SEQ ID NO: 46),
CTRXWXPVVVT (SEQ ID NO: 47),
CTKXWXPVVVT (SEQ ID NO: 48),

wherein X is any of the 20 common amino acids,

and longer peptides containing said peptides.

In one embodiment of the present invention, the peptide inhibitor of propeptide/prohormone convertase is selected from the group consisting of:

RGDVACTKQFDPVVVT (SEQ ID NO: 1),
GEDVMCPMVYDPVLLT (SEQ ID NO: 2),
DTGRVCTREYRPVTVS (SEQ ID NO: 3),
RDGVICNKLYDPVVVT (SEQ ID NO: 4),

ADGVMCTREYAPVVVT (SEQ ID NO: 5),
SEGVMCPMIYDPVLLT (SEQ ID NO: 6),
CALEGSLQKRGIVEQCC (SEQ ID NO: 7),
CALEGSLQKHGIVEQCC (SEQ ID NO: 8),
CRDGVICNKLYDPVVVTC (SEQ ID NO: 9),
CADGVMCTREYAPVVVTC (SEQ ID NO: 10),
CDTGRVCTREYRPVTVSC (SEQ ID NO: 11),
CRGDVACTKQFDPVVVTC (SEQ ID NO: 13),
CGEDVMCPMVYDPVLLTC (SEQ ID NO: 14),
CSEGVMCPMIYDPVLLTC (SEQ ID NO: 15),
RDGVICNKNYDPVVVT (SEQ ID NO: 16),
DDAVMCTREYAPVVVT (SEQ ID NO: 17),
DQDRACIKIYDPLVVT (SEQ ID NO: 18),
DAGVMCTREYAPVVVT (SEQ ID NO: 19),
RDGVMCTKQYDPVVVT (SEQ ID NO: 20),
EPGRMCTKEWRPITVT (SEQ ID NO: 21),
RDDVWCNKLYDPVVVT (SEQ ID NO: 22),
EDSVMCTREYAPVVVT (SEQ ID NO: 23),
TGDVMCTKQYDVVVT (SEQ ID NO: 24),

and longer peptides containing said peptides, such as SEQ ID NO: 25; SEQ ID NO: 26; SEQ ID NO: 27; SEQ ID NO: 28; SEQ ID NO: 29; SEQ ID NO: 30; SEQ ID NO: 31; SEQ ID NO: 32; SEQ ID NO: 33; SEQ ID NO: 34; SEQ ID NO: 35; SEQ ID NO: 36; SEQ ID NO: 37; SEQ ID NO: 38; SEQ ID NO: 39; SEQ ID NO: 40; SEQ ID NO: 41; or SEQ ID NO: 42.

In another embodiment of the present invention, the peptide inhibitor has the following sequence:

ASLYAPSALVLTVGHGTSAAAA
SPLRAVTLNCAPTASGTHPAPALA
CADLRGVGGDIDALKARDGVICNKL

YDPVVVTVDGVWQGKRVSYERTFGNE

CVKNSYGTSLFAF, (SEQ ID NO:25)

and derivatives and mutants thereof.

In another embodiment of the present invention, the propeptide/prohormone convertase is selected from the group consisting of PC1/3, PC2, PC6A, PC7 and furin.

In another embodiment of the present invention, the peptide inhibitor is useful in the treatment of a tumor cell. The tumor cell may be a hormone-secreting tumor cell, a tumor cell of a neuroendocrine neoplastic disease, or a tumor cell of a neoplastic disease involving overproduction of a growth factor.

In another embodiment of the present invention, the peptide inhibitor is useful in the treatment of a cell infected with a viral pathogen, or in the treatment of a cell infected with HIV.

In another embodiment of the present invention, a method of suppressing propeptide/prohormone convertase activity comprises the steps of treating a propeptide/prohormone convertase with a peptide selected from the group consisting of

CTRXYXPVVVT (SEQ ID NO: 43),

CTKXYXPVVVT (SEQ ID NO: 44),

CNRXYXPVVVT (SEQ ID NO: 45),

CNKXYXPVVVT (SEQ ID NO: 46),

CTRXWXPVVVT (SEQ ID NO: 47),

CTKXWXPVVVT (SEQ ID NO: 48),

wherein X is any of the 20 common amino acids,

and longer peptides containing said peptides.

In another embodiment of the present invention, a method of suppressing propeptide/prohormone convertase acitivity comprises the steps of treating a propeptide/prohormone convertase with a peptide selected from the group consisting of

RGDVACTKQFDPVVVT (SEQ ID NO: 1),
GEDVMCPMVYDPVLLT (SEQ ID NO: 2),
DTGRVCTREYRPVTVS (SEQ ID NO: 3),
RDGVICNKLYDPVVVT (SEQ ID NO: 4),
ADGVMCTREYAPVVVT (SEQ ID NO: 5),
SEGVMCPMIYDPVLLT (SEQ ID NO: 6),
CALEGSLQKRGIVEQCC (SEQ ID NO: 7),
CALEGSLQKHGIVEQCC (SEQ ID NO: 8),
CRDGVICNKLYDPVVVTC (SEQ ID NO: 9),
CADGVMCTREYAPVVVTC (SEQ ID NO: 10),
CDTGRVCTREYRPVTVSC (SEQ ID NO: 11),
CRGDVACTKQFDPVVVTC (SEQ ID NO: 13),
CGEDVMCPMVYDPVLLTC (SEQ ID NO: 14),
CSEGVMCPMIYDPVLLTC (SEQ ID NO: 15),
RDGVICNKNYDPVVVT (SEQ ID NO: 16),
DDAVMCTREYAPVVVT (SEQ ID NO: 17),
DQDRACIKIYDPLVVT (SEQ ID NO: 18),
DAGVMCTREYAPVVVT (SEQ ID NO: 19),
RDGVMCTKQYDPVVVT (SEQ ID NO: 20),
EPGRMCTKEWRPITVT (SEQ ID NO: 21),
RDDVWCNKLYDPVVVT (SEQ ID NO: 22),
EDSVMCTREYAPVVVT (SEQ ID NO: 23),
TGDVMCTKQYDVVVT (SEQ ID NO: 24),

and longer peptides containing said peptides, such as SEQ ID NO: 25; SEQ ID NO: 26; SEQ ID NO: 27; SEQ ID NO: 28; SEQ ID NO: 29; SEQ ID NO: 30; SEQ ID NO: 31; SEQ ID NO: 32; SEQ ID NO: 33; SEQ ID NO: 34; SEQ ID NO: 35; SEQ ID NO: 36; SEQ ID NO: 37; SEQ ID NO: 38; SEQ ID NO: 39; SEQ ID NO: 40; SEQ ID NO: 41; or SEQ ID NO: 42;

and monitoring the degree of inhibition of propeptide/prohormone convertases.

In another embodiment of the present invention, the propeptide/prohormone convertase is selected from the group consisting of PC1/3, PC2, PC6A, PC7, and furin.

In another embodiment of the present invention, a method of treating a tumor cell comprises the steps of treating a tumor cell with at least one peptide selected from the group consisting of

CTRXYXPVVVT (SEQ ID NO: 43),
CTKXYXPVVVT (SEQ ID NO: 44),
CNRXYXPVVVT (SEQ ID NO: 45),
CNKXYXPVVVT (SEQ ID NO: 46),
CTRXWXPVVVT (SEQ ID NO: 47),
CTKXWXPVVVT (SEQ ID NO: 48),

wherein X is any of the 20 common amino acids,

and longer peptides containing said peptides.

In another embodiment of the present invention, a method of treating a tumor cell comprises the steps of treating a tumor cell with at least one peptide selected from the group consisting of

RGDVACTKQFDPVVVT (SEQ ID NO: 1),
GEDVMCPMVYDPVLLT (SEQ ID NO: 2),
DTGRVCTREYRPVTVS (SEQ ID NO: 3),
RDGVICNKLYDPVVVT (SEQ ID NO: 4),
ADGVMCTREYAPVVVT (SEQ ID NO: 5),
SEGVMCPMIYDPVLLT (SEQ ID NO: 6),
CALEGSLQKRGIVEQCC (SEQ ID NO: 7),
CALEGSLQKHGIVEQCC (SEQ ID NO: 8),
CRDGVICNKLYDPVVVTC (SEQ ID NO: 9),
CADGVMCTREYAPVVVTC (SEQ ID NO: 10),
CDTGRVCTREYRPVTVSC (SEQ ID NO: 11),
CRGDVACTKQFDPVVVTC (SEQ ID NO: 13),

CGEDVMCPMVYDPVLLTC (SEQ ID NO: 14),
CSEGVMCPMIYDPVLLTC (SEQ ID NO: 15),
RDGVICNKNYDPVVVT (SEQ ID NO: 16),
DDAVMCTREYAPVVVT (SEQ ID NO: 17),
DQDRACIKIYDPLVVT (SEQ ID NO: 18),
DAGVMCTREYAPVVVT (SEQ ID NO: 19),
RDGVMCTKQYDPVVVT (SEQ ID NO: 20),
EPGRMCTKEWRPITVT (SEQ ID NO: 21),
RDDVWCNKLYDPVVVT (SEQ ID NO: 22),
EDSVMCTREYAPVVVT (SEQ ID NO: 23),
TGDVMCTKQYDVVVT (SEQ ID NO: 24),

and longer peptides containing said peptides; such as SEQ ID NO: 25; SEQ ID NO: 26; SEQ ID NO: 27; SEQ ID NO: 28; SEQ ID NO: 29; SEQ ID NO: 30; SEQ ID NO: 31; SEQ ID NO: 32; SEQ ID NO: 33; SEQ ID NO: 34; SEQ ID NO: 35; SEQ ID NO: 36; SEQ ID NO: 37; SEQ ID NO: 38; SEQ ID NO: 39; SEQ ID NO: 40; SEQ ID NO: 41; or SEQ ID NO: 42;

and monitoring the degree of inhibition of propeptide/prohormone convertase.

In another embodiment of the method of the present invention, the propeptide/prohormone convertase is PC1/3 or PC2.

In another embodiment of the method of the present invention, the tumor cell is a hormone-secreting tumor cell, a tumor cell of a neuroendocrine neoplastic disease, or a tumor cell of a neoplastic disease involving overproduction of a growth factor.

In another embodiment of the present invention, there is a method for treating a cell infected with a viral pathogen with at least one peptide from the group consisting of

CTRXYXPVVVT (SEQ ID NO: 43),
CTKXYXPVVVT (SEQ ID NO: 44),
CNRXYXPVVVT (SEQ ID NO: 45),

CNKXYXPVVVT (SEQ ID NO: 46),

CTRXWXPVVVT (SEQ ID NO: 47),

CTKXWXPVVVT (SEQ ID NO: 48),

wherein X is any of the 20 common amino acids,

and longer peptides containing said peptides.

In another embodiment of the present invention, there is a method of treating a cell infected with a viral pathogen with at least one peptide from the group consisting of

RGDVACTKQFDPVVVT (SEQ ID NO: 1),

GEDVMCPMVYDPVLLT (SEQ ID NO: 2),

DTGRVCTREYRPVTVS (SEQ ID NO: 3),

RDGVICNKLYDPVVVT (SEQ ID NO: 4),

ADGVMCTREYAPVVVT (SEQ ID NO: 5),

SEGVMCPMIYDPVLLT (SEQ ID NO: 6),

CALEGSLQKRGIVEQCC (SEQ ID NO: 7),

CALEGSLQKHGIVEQCC (SEQ ID NO: 8),

CRDGVICNKLYDPVVVTC (SEQ ID NO: 9),

CADGVMCTREYAPVVVTC (SEQ ID NO: 10),

CDTGRVCTREYRPVTVSC (SEQ ID NO: 11),

CRGDVACTKQFDPVVVTC (SEQ ID NO: 13),

CGEDVMCPMVYDPVLLTC (SEQ ID NO: 14),

CSEGVMCPMIYDPVLLTC (SEQ ID NO: 15),

RDGVICNKNYDPVVVT (SEQ ID NO: 16),

DDAVMCTREYAPVVVT (SEQ ID NO: 17),

DQDRACIKIYDPLVVT (SEQ ID NO: 18),

DAGVMCTREYAPVVVT (SEQ ID NO: 19),

RDGVMCTKQYDPVVVT (SEQ ID NO: 20),

EPGRMCTKEWRPITVT (SEQ ID NO: 21),

RDDVWCNKLYDPVVVT (SEQ ID NO: 22),

EDSVMCTREYAPVVVT (SEQ ID NO: 23),

TGDVMCTKQYDVVVT (SEQ ID NO: 24),

and longer peptides containing said peptides; such as SEQ ID NO: 25; SEQ ID NO: 26; SEQ ID NO: 27; SEQ ID NO: 28; SEQ ID NO: 29; SEQ ID NO: 30; SEQ ID NO: 31; SEQ ID NO: 32; SEQ ID NO: 33; SEQ ID NO: 34; SEQ ID NO: 35; SEQ ID NO: 36; SEQ ID NO: 37; SEQ ID NO: 38; SEQ ID NO: 39; SEQ ID NO: 40; SEQ ID NO: 41; or SEQ ID NO: 42;

and monitoring the degree of inhibition of propeptide/prohormone convertase.

In another embodiment of the method of treating cells infected with a viral pathogen, the propeptide/prohormone is PC6A, PC7, or furin.

In another embodiment of the method of treating cells infected with a viral pathogen, the cell is selected from the group consisting of lymphoid and monocyte/macrophage cells.

Another embodiment of the present invention relates to the nucleic acid encoding the peptide inhibitors of the present invention.

In another embodiment of the present invention, an expression vector for a peptide inhibitor comprises at least one nucleic acid encoding at least one peptide inhibitor of the present invention, operatively linked to a nucleic acid encoding a signal peptide.

In another embodiment of the present invention, the expression vector of the present invention comprises a nucleic acid encoding a Golgi retention signal.

In another embodiment of the present invention, the expression vector of the present invention further comprises a nucleic acid encoding an inducible element.

In another embodiment of the present invention, the inducible element of the expression vector of the present invention is a 5' LTR component of HIV-1.

Another embodiment of the present invention relates to a chimeric protein comprising at least one peptide inhibitor of the present invention, fused at its amino terminus to a signal peptide.

Another embodiment of the present invention relates to an expression vector for a peptide inhibitor comprising at least one nucleic acid encoding at least one peptide of the present invention, operatively linked to a nucleic acid encoding a carrier peptide.

In another embodiment of the present invention, the carrier peptide is a preproregion of a prohormone.

In another embodiment of the present invention, the preprohormone is prosomatostatin.

In another embodiment of the present invention, the expression vector further comprises a nucleic acid encoding a Golgi retention signal.

In another embodiment of the present invention, the expression vector further comprises a nucleic acid encoding an inducible element, which may be a 5' LTR of HIV.

Another embodiment of the present invention relates to a chimeric protein comprising at least one peptide inhibitor of the present invention, fused at its amino terminus to the preproregion of a prohormone.

Other features of the invention will become apparent in the course of the following descriptions of exemplary embodiments which are given for illustration of the invention and are not intended to be limiting thereof.

Researchers have established that most bioactive peptides used by the endocrine, nervous and immune systems are initially synthesized as larger precursors. These precursors must be proteolytically cleaved by endoproteases to release their bioactive peptide products. The existence of mammalian endoproteases capable of propeptide processing has been known for some time. Examples include enzymes involved in the conversion of precursors for peptide hormones, growth factors, receptors for both peptide hormones and growth factors, envelope glycoproteins of viral pathogens, and toxins. The enzymes that process biologically inactive precursors to release bioactive products are referred to as propeptide/prohormone convertases

(PCs). PCs are generally specific for cleaving at pairs of basic amino acid residues (for example Lys-Arg), although PCs can also cleave at sites containing only one basic amino acid (for example Arg) or at sites with more than two basic amino acids. Identification and characterization of the PCs was difficult, because their low abundance and the lack of specific assays made it difficult to separate the PCs from more abundant and nonspecific proteases which contaminated sample preparations.

Examination of a mutant strain of *Saccharomyces cerevisiae* revealed Kex2, a cleavage enzyme with a catalytic region which is 28% identical with the bacterial subtilisin serine proteases which cleave at the C-terminus of both Lys-Arg and Arg-Arg pairs with approximately equal efficiency, but cleave poorly after single arginine residues or at pairs of lysines. The discovery and characterization of Kex2 represents the first identification of an authentic propeptide/prohormone convertase. Subsequently, a mammalian homolog of Kex2, furin, was identified.

Employing polymerase chain reaction (PCR) methodology with primers designed to amplify the conserved active site regions of both Kex2 and furin, two different enzymes were characterized and identified as candidate prohormone convertases. Primers directed at the active site His/Asp within Kex2 were used to identify and characterize PC2. A putative Kex2-like endoprotease, referred to as PC1, was cloned using primers based on the Asp/Ser site in furin. The same cDNA sequence was obtained by another research group who called this member of the family PC3. Here the term PC1/3 is used for all references to this enzyme. PC1/3 and PC2 share 55% homology with each other and approximately 50% homology with Kex2 in their catalytic domains.

The PC mRNAs encode proteins which, while highly homologous to furin, exhibit some distinct differences. The predicted size of PC1 /3 is 87 kDa while PC2 is expressed as either a 66 kDa or a 64-66 kDa form. The PCs do not contain the cysteine-rich region, the transmembrane domain, or the cytoplasmic tail observed in furin. The catalytic domain contains the subtilisin-like active site Asp, His, Asn (Asp for PC2), and Ser residues. Both PC1/3 and PC2, like Kex2 and furin, contain a

hydrophobic N-terminal signal peptide which is cleaved in the endoplasmic reticulum. Both the N-terminal and the C-terminal extensions of the catalytic region are required for proper folding and trafficking through the regulated secretory pathway. Within the P-domain there is a conserved RGD sequence, the function of which is unknown. This sequence may interact with integrin-like proteins on the cell surface after exocytosis. The C-terminal segment of the convertases is the most variable domain among the different members of the PC family. At the C-terminus of both PC1/3 and PC2, there is an amphipathic C-terminal helix. Some of the similarities and differences among several of the known converting enzymes and a comparison with Subtilisin BPN' are highlighted in Figure 1.

Figure l provides a diagrammatic representation of some of the known members of the furin/PC family along with their tissue distribution. Filled boxes represent signal peptide; arrows, pro-enzyme cleavage site; diagonal shading, domain with subtilisin homology showing the location of residues corresponding to active site amino acids in subtilisin BPN'; STR, serine-threonine rich domain; dotted boxes, cysteine rich region; cross-hatched area, transmembrane domain (TM); AH, amphipathic helix.

The cleavage specificities of PC1/3 and PC2 have been studied either by co-expression of these convertases with precursor substrates and defining the processed products by immunoprecipitation, or by *in vitro* incubations of a number of peptide substrates with either recombinant or immunopurified forms of PC1/3 or PC2. The results of these studies revealed that both enzymes selectively cleaved various precursors at distinct pairs of basic residues. Data generated using prodynorphin demonstrated that both convertases are also capable of cleaving at a single Arg residue. From the available data, it is clear that only PC1/3, but neither PC2 nor furin, can cleave substrates with an aliphatic residue (leucine, valine, or isoleucine) immediately C-terminal to the cleavage site. It is also evident that the presence of a basic residue (most often Arg) at positions -4, -6, or -8 from the cleavage site favors processing at the basic residue at position -1 by PC1/3, PC2, and furin. In general, the

available cleavage specificity data demonstrate that PC1/3 and PC2 cleave precursors both at specific single basic residues and at pairs of basic residues, and that the four combinations Lys-Arg, Arg-Arg, Lys-Lys, and Arg-Lys are cleavage sites for these convertases.

Because of active site homology of the PCs to subtilisin BPN', the present inventors investigated naturally occurring inhibitors of the subtilisin BPN' proteases produced by the gram positive bacteria Streptomyces as possible PC inhibitors. Streptomyces Subtilisin Inhibitor (SSI), which was originally purified from the culture medium of the bacterial strain *Streptomyces albogriseolus* S-3253, exhibits a strong inhibitory preference toward the subtilisin BPN' proteinase (the putative ancestor of the mammalian PCs). See S. Murao et al.(1972) *Agric. Biol. Chem.* 36:160-163 and S. Murao et al. (1972) *Agric. Biol. Chem.* 36:1737-1744. Subsequently, numerous other representatives of the SSI family have been identified and characterized See S. Obata et al. (1989) *J. Biochem.* 105:367-371; S. Obata et al. (1989) *J. Biochem.* 105:372-376; Y. Ueda et al. (1992) *J. Biochem.* 112:204-211; S. Taguchi et al. (1993) *Applied and Environmental Microbiology* 4338-4341; M. Terabe et al. (1994) *Eur. J. Biochem.* 226:627-632; S. Taguchi et al. (1992) *FEMS Microbiology Letters* 99:293-298; S. Kojima et al. (1994) *Biochim. Biophys. Acta* 1207:120-125; S. Takaguchi et al. (1994) *Eur. J. Biochem.* 220:911-918; and M. Terabe et al. (1994) *J. Biochem.* 116:1156-1163 . All of the SSI inhibitors characterized to date exist as dimeric proteins consisting of two identical subunits connected by disulfide bridges. The specificity and kinetics of the SSI inhibitors reside in the amino acid sequence surrounding the reactive site. SSI-like inhibitors function by forming complexes with the target protease but cleavage of the peptide bond at the reactive site of the inhibitor is prevented. The reactive site of the SSI inhibitor is comprised of the amino acid pair Met(73)-Val(74), whereas in the similar STI-1 and STI-2 inhibitors the reactive sites are comprised of Arg-Glu and Lys-Leu, respectively. See J.E. Strickler et al. (1992) *J. Biol. Chem.* 267:3236-3241. All SSI-like inhibitors exhibit a strong inhibitory preference for the subtilisin-like over the trypsin-like

proteinases. The PCs are specific for cleavage at either monobasic or dibasic amino acid cleavage sites. It is intriguing that in the SSI homologs STI-1 and STI-2, and in 16 additional SSI-like inhibitors, the first of the two inhibitor reactive site residues is a basic amino acid.

To identify and elucidate the characteristics of PC inhibition, a method for assessing the physiologic mediators of proglucagon cleavage was developed. Using the αTC1-6 glucagonoma cell line as a model system, an *in vitro* assay protocol was developed that employed HPLC purified ^{3}H-tryptophan labeled proglucagon incubated with recombinant PC1/3 or immunopurified PC2 to determine their cleavage specificities.

Several criteria were employed in choosing an appropriate model cell system. The first criterion was to select a mammalian endocrine cell line which synthesizes one or more precursor derived peptides and which co-expresses one or more of the known PCs. Such a culture system permits examination of the function of the PCs in relation to their substrates in intact cells. Second, the cell line should express a prohormone substrate which undergoes differential processing involving the activities of multiple PCs. The αTC1-6 cell line fulfills these criteria.

The αTC1 cell line evolved from a glucagonoma developed in transgenic mice. The mice express a hybrid gene consisting of a glucagon-promoter sequence fused to the SV40 T-antigen oncoprotein. The αTC1-6 line was shown to maintain many of its differentiated pancreatic α-cell characteristics for greater than 40 passages and expresses high levels of preproglucagon mRNA. Radioimmunoassays were used to demonstrate that these cells synthesize large quantities of glucagon, some glucagon-like peptide-I (GLP-I), and small amounts of unprocessed proglucagon. This cell line also expresses PC1/3, PC2, and PC6A.

Proglucagon is an excellent substrate to use in the proposed studies because of the potential for differential processing. In fact, multiple PCs are involved in proglucagon processing in the αTC1-6 cell. Moreover, performance of the proposed experiments using a tumor cell line affords the opportunity to test inhibitors

in an environment that mimics cellular conditions in neoplastic tissue. Naturally occurring glucagonomas secrete hyperphysiologic amounts of glucagon and GLP-I which leads to an imbalance in the homeostatic regulation of blood glucose levels Accordingly, the αTC1-6 cell line provides an ideal model system in which to test the efficacy of broad spectrum PC inhibitors.

Further, vectors encoding antisense DNA sequences were transfected into the αTC1-6 cells to inhibit expression of PC1/3 and PC2. Sensitive RIAs were developed and employed to determine the relative reduction in PC levels induced by the antisense transfections (~40% for PC1/3 and ~91% for PC2). Four days after transient transfection of αTC1-6 cells, ^{3}H-tryptophan was incorporated. After a 3 hr incubation, cells were extracted and the extracts subjected to HPLC analyses. Proglucagon and its cleavage products were recovered and subjected to peptide mapping which allowed accurate quantification of product/precursor ratios. These data demonstrated that PC1/3 is capable of participating in essentially all of the potential cleavage events in processing proglucagon, whereas participation of PC2 is limited to activity at only two of the four possible cleavage sites.

To examine further the potential roles of the PCs in proglucagon processing, chemically synthesized inhibitors were tested in the *in vitro* assay. The inhibitors, peptidyl derivatives of (α-aminoalkyl) phosphonate diphenyl esters were designed to specifically inhibit subtilisin-like serine proteinases. Recombinant PC1/3 or immunopurified PC2 were incubated ±50 μM inhibitor with HPLC purified ^{3}H-tryptophan labeled proglucagon derived from metabolically labeled αTC1-6 cells. After incubation, the reaction mixtures were subjected to RP-HPLC to separate proglucagon cleavage products. Two of the inhibitors tested, Z-Thr-(4-AmphGly)^{P}(oph)$_2$ (Figure 2) and BOC-Leu-Thr-(4-AmphGly)^{P}(oph)$_2$, completely blocked PC1/3 mediated processing of mouse proglucagon but had no apparent effect on PC2 activity. One other inhibitor, Z-Ala-Ala-Ala ^{P}(oph)$_2$, totally inhibited the action of both PC1/3 and PC2. The inhibitor 2HCl (4-AmphGly)^{P}(oph)$_2$ had no obvious effect on the processing of the precursor by either recombinant PC1/3 or

immunopurified PC2. These results provide verification that the *in vitro* proglucagon conversion assay can be employed to determine the effects of inhibitors on PC activity. Representative results from this type of experiment are depicted in Figures 2A and 2B.

Figures 2A and 2B depict reversed phase HPLC chromatograms from *in vitro* inhibitor studies utilizing recombinant PC1/3 (A) or PC2 (B), respectively, ±50 μM of Z-Thr-(4-AmphGly)^{P}(oph)$_2$ as the inhibitor, to determine the effects of the inhibitor on processing of proglucagon. Products are: 1 - glicentin, 2 - oxyntomodulin, 3 - glucagon, 4 - GLP-I(1-36) amide/(7-36) amide, 5 - proglucagon 54/55, 6 - proglucagon 56, 7 - proglucagon 58 (the three forms of proglucagon are differentially methionine sulfoxidized), and 8 - MPGF. Solid lines with open circles depict PC incubations with the inhibitor, dashed lines with solid circles depict PC incubations without the inhibitor.

However, for these phosphonate diphenyl ester PC inhibitors to be effective in any possible therapeutic approach aimed at reducing PC activity in intact cells, they would have to pass through the plasma membranes of cells and enter secretory granules in quantities sufficient to impair processing activity. To test the possibility that such synthetic inhibitors might be effective in diminishing PC activity in intact cells, αTC1-6 cells were incubated overnight in the presence of either Z-Thr-(4-Amph-Gly)^{P}(oph)$_2$ or BOC-Leu-Thr-(4-Amph-Gly)^{P}(oph)$_2$ at concentrations up to 250 μM (higher concentrations were toxic to the cells). After a 2.5 hr pulse incubation with radiolabeled tryptophan, cells were extracted and generation of proglucagon cleavage products was assessed by HPLC and liquid scintillation spectroscopy. The results demonstrated that the cells incubated with these inhibitors generated quantities and proportions of cleavage products which did not differ from those produced by cells incubated for the same period of time in the absence of inhibitors. These results suggested that, even though these inhibitors are quite hydrophobic, insufficient amounts entered the secretory granules to impair PC cleavage activity.

These results indicate that it is necessary to introduce the PC inhibitors directly into secretory granules to effectively inhibit PC function in intact cells.

EXAMPLES:

Experiments were then undertaken to identify peptide PC inhibitors. Recombinant PC1/3 or PC2 was incubated with a fluorogenic substrate ± the following four synthetic peptides:

hProinsulin C-A(KR) H_2N-ALEGSLQ**KR**GIVEQCC-COOH (normal) (SEQ ID NO: 49)

hProinsulin C-A(KH) H_2N-ALEGSLQ**KH**GIVEQCC-COOH (mutant) (SEQ ID NO: 50)

STI-2 H_2N-RDGVICN**KL**YDPVVVT-COOH (SSI-like) (SEQ ID NO: 4)

STI-2(KN) H_2N-RDGVICN**KN**YDPVVVT-COOH (SSI-like) (SEQ ID NO: 16)

SIL-2 H_2N-ADGVMCT**RE**YAPVVVT-COOH (SSI-like) (SEQ ID NO: 5)

Plasminostreptin (PSN) H_2N-RGDVACT**KQ**FDPVVVT-COOH (SSI-like) (SEQ ID NO: 1)

SSI H_2N-GEDVMCP**MV**YDPVLLT-COOH (Original SSI) (SEQ ID NO: 2)

API-2C H_2N-SEGVMCP**MI**YDPVLLT-COOH (SSI-like) (SEQ ID NO: 6)

The cleavage inhibitor reactive sites are indicated in bold type and underlined.

These synthetic peptides, as well as the other peptides pertaining to the present invention, were synthesized in accordance with known techniques, exemplified, for example, by the Merrifield solid-phase synthesis described in techniques in J.M. Stewart and J.D. Young: Solid-Phase Peptide Synthesis, Freeman, San Francisco, 1969, which is incorporated herein by reference.

The full length inhibitors were obtained by means of expression and purification of full length SSI-like inhibitors in a prokaryotic expression system. DH5α cells were transformed with the inducible plasmid vectors containing the wild type

STI-2 coding sequence (pOSTI-2) or a mutated sequence which encodes Lys (pOSTI-2[N72K]) or Arg(pOSTI-2[N72R]) in place of Asn immediately upstream of the reactive site. The host bacteria are then cultured in the presence of 100 μg/ml ampicillin and 1 mM IPTG for 16 hrs. Cells are collected by centrifugation and lysed by repeated freeze-thaw in phosphate buffered saline. Soluble components are then dialyzed against water followed by partial purification using a concentrator with a molecular weight cutoff of 10,000. The inibitor proteins were further purified by reversed phase HPLC. At a flow rate of 4 ml/min a 40 min linear gradient is run from 25% A (A is 80% acetonitrile/0.1% TFA; B is 0.1% TFA) to 44% A, then holding a 44% A for 10 min. Fractions were analyzed for STI-2 immunoreactivity using SDS PAGE/ western analysis with a polyclonal SSI-antibody. Immunoreactive fractions were pooled and lyophilized, followed by resuspension in sterile water for inhibitor studies.

Procedures were also developed for expression, purification, and testing the activity of full-length PC1/3, PC2, and PC6A. The Baculogold DNA reagent system (PharMingen) was utilized. The cDNA for mouse PC1/3 was cloned into the plasmid pVL1392. This plasmid was then co-transfected into Sf9 insect cells along with Baculovirus DNA (Baculogold DNA) from which an essential part of the Baculovirus genome had been removed. Co-transfection of the plasmid and the altered genomic DNA was facilitated by lipofection (Lipofectin, Gibco/BRL). The mPC1/3/pVL1392 plasmid construct contained the missing sequence of the Baculovirus genome. After homologous recombination of the cloned insert DNA into the compromised Baculovirus genomic DNA, copies of virus were produced by the Sf9 cells. Theoretically, only those pieces of genomic DNA which have undergone homologous recombination are capable of producing viable virus. The resulting budded viral particles were then collected from the cell culture media and amplified through a second round of infection in Sf9 cells. Second round infection generally resulted in viral titers of ~1 x 10^8 plaque-forming units/ml. The PC1/3-containing virus were used to infect fresh cultures of High Five cells (Invitrogen) at a multiplicity of

infection of approximately 10 viruses per cell. After a 72 hour infection, media were collected and samples subjected to buffer exchange by passing them through a Sephadex G-25 column. For control experiments, media from High Five cells infected with the wild-type virus, and from cells not exposed to viral infection, were also collected and treated in parallel.

To estimate sizes of expressed products, proteins from culture samples were precipitated and subjected to SDS PAGE. The separated proteins were then electroblotted onto polyvinylidene difluoride (PVDF) membranes and PC1/3 proteins were detected by antibody staining. Routinely, two PC1/3-related proteins with approximate molecular weights of 80 and 88 kDa were detected using this procedure. It is probable that the larger molecular weight form represents the precursor to the active 80 kDa enzyme. Samples were also tested for proteolytic activity using the substrate Pyr-Arg-Thr-Lys-Arg-AMC. Only the media from the PC1/3-virus infected cells contained proteolytic activity capable of recognizing and cleaving this substrate. These results indicate that active mPC1/3 has been produced using the Baculovirus protein expression system The cDNAs for mPC2 and mPC6A have also been used to generate recombinant Baculoviruses. Employing procedures substantially similar, active recombinant PC2 and PC6A have been generated. The molecular sizes of the primary products are 75 kDa (precursor) and 68 kDa (active form) for PC2 and 95 kDa (precursor) and 60 kDa (active form) for PC 6A.

To verify that the recombinant PC1/3 and PC2 will cleave a natural substrate, assays for recombinant PC activity were also performed using recombinant human proinsulin purchased from Sigma. Proinsulin (5 μg) was incubated with 10 to 25 μl of the recombinant PC preparation with 5 mM calcium acetate at pH 5.5 for 4 hr at 37°C. Reactions were terminated by freezing the samples on dry ice. Prior to HPLC analyses, proinsulin and its cleavage products were reduced and alkylated to remove disulfide bonds and block all cysteine residues. Reaction mixtures were diluted in concentrated Tris buffer, pH 8.7, containing 0.5 mM EDTA, 8 M urea (reducing and alkylating buffer) and DTT at a final concentration of 57.5 mM. Samples were

sparged with argon gas and incubated at 50°C for 30 min. After cooling to ambient temperature, reducing and alkylating buffer containing sufficient iodoacetate to achieve a final concentration of 95 mM was added and the samples incubated for 7 min protected from light at ambient temperature. Reactions were terminated by adding glacial acetic acid to achieve a final concentration of 3.5 M. To assess cleavage activity, the samples were then subjected to HPLC in TFA/acetonitrile over a narrow bore Vydac C4 column. Proinsulin and processing intermediates in the HPLC eluates were monitored by absorbance at 210 nm. The B-chain+C peptide and C-peptide+A-chain intermediates were readily differentiated with this procedure. Cleavage efficiency was estimated by comparing amounts of intact proinsulin vs. intermediate(s). These conversion experiments indicated that the recombinant PC1/3 is capable of cleaving at the B-chain/C-peptide junction to release the B chain from the remainder of the precursor. Results from conversion assays in which the active form of recombinant PC2 was combined with proinsulin confirmed that cleavage occurred appropriately at the proinsulin C-A cleavage site.

The recombinant enzymes are further purified to eliminate any contaminating activity. A purification protocol was developed, and purification of PC1/3 was performed as an example. Specifically, a PC1/3 concentrate was prepared using an Amicon Centriprep concentrator having a 30,000 molecular weight cut-off. The concentrate was then subjected to FPLC at 4°C on a Phamacia Hi Trap SP column with an elution buffer of 20mM sodium acetate/acetic acid, 1mM 2-mercaptoethanol at pH 5. Prior to loading, the sample was preincubated in 5 volumes of elution buffer for 1 hour at 37°, to permit autocatalysis to remove the propeptide and C-terminal extension peptide. After loading the sample, the column was washed for 5 minutes and then eluted by applying a linear gradient of 0-200 mM sodium sulfate over 30 minutes at a flow rate of 1ml/min. One minute fractions were collected in tubes containing sufficient glycerol to achieve a final concentration of 20%. Aliquots of 25 µl from each tube are tested in the fluorometric assay to determine enzyme activity. Samples were examined by SDS PAGE on duplicate gels, followed by Coomaassie staining and

western analysis using a PC1/3 antibody at a concentration of 1:1000. The preparation was devoid of any activity other than that contributed by the PC.

An *in vitro* fluorometric assay was used to test the inhibitor peptides for their efficacy in blocking PC-mediated cleavage. The fluorometric assays consisted of incubations at 37°C of enzyme ± putative inhibitor in 100 mM sodium acetate buffer, pH 5.2, with 5 mM calcium acetate and 200 μM fluorogenic substrate (Pyr-Arg-Thr-Lys-Arg-AMC). This substrate contains the fluorescent group 7-amino-4-methyl-coumarin (AMC) which is about 500 times more fluorescent after its release from the peptide portion of the parent molecule. PC-mediated cleavage occurs at the Arg-AMC bond. Substrate and inhibitor were added to the other components at the beginning of each incubation. Either the entire reaction mixture, or diluted aliquots, were monitored at 15 min intervals in a fluorometer with the excitation wavelength set at 380 nm and the emission wavelength set at 460 nm. The results are depicted in Figures 3, 4A - 4G, 5, 6A - 6D, and 7A - 7E.

Modifications in the *in vitro* fluorometric assay are used to test the peptide and full length inhibitors for their efficacy in blocking PC-mediated cleavage, respecting furin, PC6A and PC7. The ability of furin, PC6A and PC7 to cleave either the fluorogenic substrate pGlu-Arg-Thr-Lys-Arg-AMC or the fluorogenic substrate pArg-Thr-Lys-Arg-AMC is compared. The fluorometric inhibitor assays are performed as previously described, except that the incubations for furin and PC6A are performed at pH 7.5, while those for PC7 are performed at pH 6.5. Varying concentrations of each inhibitor peptide are analyzed to determine effective dose ranges. The substrate most efficiently cleaved is then used to screen the ability of inhibitors to block PC activity. The ability of recombinant furin, PC6A and PC7 to cleave natural substrates, such as proinsulin or progucagon or gp160, is then tested.

Figure 3 depicts the effects of natural and Streptomyces inhibitor peptides on the ability of PC1/3 to cleave a fluorogenic substrate. Control incubations were performed with substrate plus enzyme only. Relative fluorescence indicated on the Y-axis is in arbitrary units read directly from the fluorometer display at 15 minute

intervals. The results demonstrate that PC1/3 was found to be much less susceptible than PC2 to the action of each of these inhibitor peptides. The effects on PC1/3 activity are shown in Figure 3. For the experiment shown, the inhibitor concentration was 200 μM. Clearly, PC1/3 is partially inhibited by any of these peptides at this peptide concentration.

The results from incubation of these peptides with PC2 were strikingly different. The two peptides hypothesized to be inhibitors, SIL-2 and STI-2, each functioned as very effective inhibitors of PC2 at final concentrations of either 4 μM or 40 μM (Figures 4A-4G). Inhibitor potency tests yielded IC_{50} values of about 30 μM and 10 μM for STI-2 activity against PC1/3 and PC2, respectively. The peptide proinsulin C-A(KR) had been anticipated to serve as a "control" for the other inhibitors because it was expected to be cleaved by PC2, but it was also quite effective in inhibiting PC2 activity (Figure 4G). This probably is the result of this peptide serving as a better substrate for PC2 than the fluorogenic substrate. Each of the inhibitory peptides exhibited a unique kinetic pattern when incubated with PC2 at a concentration of 40 μM (Figure 4B), indicating that each interacts with the enzyme in a different manner. The effects of the STI-2, SIL-2, and proinsulin C-A(KR) peptides on PC2 are concentration dependent (Figures 4E-4G). Inclusion of somatostatin-14, a peptide of approximately the same size as the PSN and SSI peptides but lacking the SSI-like sequence, resulted in a cleavage pattern of the fluorogenic substrate identical to that of the control.

Moreover, additional experiments demonstrated that peptides based on the sequences of the Streptomyces subtilisin inhibitors (SSI)-like inhibitors SIL-2, PSN, SSI and API-2C were either ineffective or only marginally effective PC inhibitors. However, the STI-2 based peptide generated dose-dependent (3 to 200 μM) inhibition of both PC1/3 and PC2 in the fluorometric assay and, at a concentration of 200 μM, completely inhibited both PC1/3 and PC2 mediated proglucagon conversion. The detailed protocol for the proglucagon conversion assay has been previously presented. See Rothenberg et al. (1995) *J. Biol. Chem.* 270:10136-10146,

which is incorporated herein by reference. Briefly, αTC1-6 cells are incubated with 250 μCi of ^{3}H-tryptophan for one hour, the cells are extracted, and radiolabeled proglucagon is isolated by reversed phase HPLC. After overnight incubation of the radiolabeled substrate in the presence of recombinant PC, reaction products are separated by HPLC, and radioactivity attributable to glucagon related cleavage products is quantitated by liquid scintillation spectrometry. When an inhibitor is being tested, it is incubated with the PC for 30 min prior to addition of the labeled proglucagon. The results from these assays demonstrate whether the inhibitor in question successfully competes with a natural substrate to inhibit PC activity.

Further experiments were conducted to determine the ability of full length recombinant STI-2, including two mutants, to compromise the activity of recombinant PC1/3 in the fluorometric assay. The mutants encode a Lys (STI-2[N72K]) or an Arg (STI-2[N72R]) in place of Asn immediately upstream of the reactive site. These full length inhibitors were generated from plasmids (pOSTI) containing inserts for STI-2, STI-2 [N72R] and STI-2 [N72K]. DH5α cells were transfected with these vectors and then cultured in the presence of 100 μg/ml ampicillin and 1 mM IPTG for 16 hours. Cells were collected by centrifugation and lysed by repeated freeze-thaw in phosphate buffered saline. Soluble components were dialyzed against water followed by partial purification using a Centriprep concentrator (Amicon) with a molecular weight cutoff of 10,000. Inhibitors were further purified by HPLC using a Vydac C18 semi-preparative column. At a flow rate of 4ml/min, a 40 min linear gradient was run from 25% A (A= 80% acetonitrile/0.1 TFA; B= 0.1% TFA) to 44% A, then holding at 44% A for 10 minutes. Fractions were analyzed for SSI-like immunoreactivity using SDS PAGE/western analysis with a polyclonal SSI-antibody. Immunoreactive fractions were pooled, lyophilized and resuspended in sterile water.

Two additional 16-amino-acid-long peptides based on the sequences of the Streptomyces inhibitors, plasminostreptin (PSN; reactive site amino acid pair = KQ) and SSI (reactive site amino acid pair = MV), were also tested. Figures 5, 10 and

11 depicts the results. It was determined that the full length STI-2 protein was no more effective as an inhibitor of PC1/3, PC2 or furin mediated cleavage of fluorogenic substrate than the STI-2 peptide. However, both the [N72K] and [N72R] mutant forms of the STI-2 full length protein proved to be effective inhibitors of PC2 mediated cleavage than either the STI-2 peptide or the full length STI-2 (Figure 10A-F). The STI-1 [N72R] protein is by far the most potent inhibitor of both furin and PC6A. See Figures 11A-D. This modified inhibitor protein exhibits dose dependent inhibition of both recmbinant furin and PC6A in the fluorometric assay, with substantially complete inhibition of furin at concentrations as low as 50 μM, with an IC_{50} of about 12.9 μM, also indicating its suitability for inhibition of gp160 processing enzyme.

Figure 5 depicts the comparison of the effects of full-length STI-2 and 16-amino-acid-long peptides based on the sequences of PSN and SSI on recombinant PC1/3 cleavage of a fluorogenic substrate. Conditions were the same as described for Figure 3, except that the concentration of STI-2 was 100 μM and the concentrations of the PSN and SSI peptides were 200 μM. The 30 minute lag prior to initiation of substrate cleavage occurred because the PC1/3 preparation was not preincubated to activate the enzyme. The two 16-amino-acid-long Streptomyces inhibitor peptides were relatively ineffective in blocking PC1/3 activity. However, the full length STI-2 protein proved to be a very potent inhibitor of both PC1/3 and PC/2. These results demonstrate that STI-2, either as a peptide or full length protein, is effective in blocking both PC1/3 and PC2 activity.

The full sequence of STI-2 is:

ASLYAPSALVLTVGHGTSAAAATPLRAVTLNCAPTASGTHPAPALACADLRG VGGDIDALKARDGVICNKLYDPVVVTVDGVWQGKRVSYERTFGNECVKNSY GTSLFAF. (SEQ ID NO: 25)

The sequence is described in J.E. Strickler et al. (1992) *J. Biol. Chem.* 267:3236-3241, which is incorporated herein by reference.

In addition to the peptide sequences identified above, the following peptide sequences are also considered as suitable as PC inhibitors.

SIL1 H_2N-DTGRVCT**RE**YRPVTVS-COOH (SEQ ID NO: 3),

and derivatives thereof,

as well as the peptides

CALEGSLQKHGIVEQCC (SEQ ID NO: 8)

CRDGVICNKLYDPVVVTC (SEQ ID NO: 9)

CADGVMCTREYAPVVVTC (SEQ ID NO: 10)

CDTGRVCTREYRPVTVSC (SEQ ID NO: 11)

CRDGVICNKLTDPVVVTC (SEQ ID NO: 12)

CRGDVACTKQFDPVVVTC (SEQ ID NO: 13)

CGEDVMCPMVYDPVLLTC (SEQ ID NO: 14)

CSEGVMCPMIYDPVLLTC (SEQ ID NO: 15),

and derivatives thereof.

The term derivatives shall encompass longer peptides which incorporate the peptide sequences identified. It will be apparent that some additional amino acid substitutions may increase inhibitor efficacy.

Figures 6A - 6D illustrate the effects of proinsulin C-A (KR) and proinsulin C-A(KH) peptides on the ability of PC1/3 and PC2 to cleave the fluorogenic substrate. Control incubations were performed with substrate plus enzyme only. Relative fluorescence indicated on the Y-axis is in arbitrary units derived directly from the fluorometer readings.

These experimental results indicate that the peptide proinsulin C-A(KR) is a moderately effective "partial inhibitor" of these preparations of PC2 (Figure 6B) but essentially ineffective in reducing PC1/3 activity in the fluorometric assay (Figure 6A). It is probable that proinsulin C-A(KR) peptide serves as a better substrate for PC2 than the fluorogenic substrate and therefore competes as an alternative substrate in the assay. This supposition was tested further by examining the efficacy of the proinsulin C-A(KR) peptide in preventing cleavage of proglucagon. The proinsulin C-A(KR) peptide exhibited no inhibition of proglucagon conversion mediated by either PC1/3 or PC2 suggesting that both enzymes preferentially cleave the natural substrate,

proglucagon. The second proinsulin derived peptide, proinsulin C-A(KH), proved to be ineffective as an inhibitor of either PC1/3 or PC2 at concentrations up to 200 μM in both the fluorometric (Figures 6C and 6D) and proglucagon conversion assays. These results indicate that neither of the proinsulin derived peptides is a good candidate as a PC inhibitor.

Figures 7A - 7E depict the effects of the STI-2 peptide and the modified STI-2(KN) peptide on PC1/3 and PC2 activity in the fluorometric assay. The STI-2 peptide was demonstrated to be a very effective inhibitor of both PC1/3 and PC2 activities in these reactions. As shown in Figures 7A and 7B, this peptide exhibits strong dose dependent inhibition of both of these PCs. Several experiments to determine the half-maximal inhibitory concentrations for the STI-2 peptide in the fluorometric assay were performed. The resulting IC_{50} values were 30.1 ± 2.9 μM and 10.0 ± 1.2 μM (mean ± S.D.) for PC1/3 and PC2, respectively, as illustrated in Figures 7D and 7E. The substrate concentration for the IC_{50} experiments was 200μM, indicating that the STI-2 peptide is a potent inhibitor of these PCs. Of additional note is the observation that substitution of Asn for Leu in the C-terminal position of the reactive site completely abolished the ability of the STI-2 peptide to inhibit either PC1/3 or PC2 in the fluorometric assay, as illustrated in Figure 7C.

The experiments with the proinsulin peptides demonstrated that the inhibition by proinsulin C-A(KR) peptide was "partial." This suggests either that the substrate and the inhibitor can occupy the enzyme simultaneously or that the enzyme preparations contain two enzymes catalyzing this reaction and only one is inhibited by the added peptide. Further experiments by the present inventors suggest that it is the latter. The enzyme preparations used in these studies were essentially Hi Five cell media obtained from cells expressing the PC enzymes. Control experiments with cell media from cells infected with wild type virus produced a small amount of background activity against the fluorogenic substrate. Thus, it was important to determine if the observed inhibition was due to binding to the PC enzymes or the "other" activities in the preparations.

This issue was examined by testing the STI-2 peptide as an inhibitor in the proglucagon conversion assay. Figures 8A - 8F depict the effects of the STI-2 peptide on PC1/3 or PC2 mediated proglucagon conversion *in vitro*. Reversed phase HPLC was employed to separate proglucagon cleavage products. MPGF denotes Major Proglucagon Fragment. Neither the PC1/3 nor the PC2 enzyme preparations generated random degradation of proglucagon. Incubations consistently produced identifiable proglucagon cleavage products. At a concentration of 200 μM, STI-2 exhibited essentially complete inhibition of both PC1/3 (Figures 8A - 8C) and PC2 (Figures 8D - 8F) mediated proglucagon conversion *in vitro*.

Figures 9A -9C depict the effects of STI-2(KN) on PC1/3 mediated proglucagon conversion in vitro. Figures 9D - 9E depict the effects of the SIL-2 peptide on PC1/3 and PC2 activity in the fluorometric assay. The modified STI-2(KN) peptide exhibited no capacity to inhibit either PC1/3-(Figures 9A - 9C) or PC2-mediated proglucagon conversion. In combination, these results demonstrate that the STI-2 peptide acts as a potent inhibitor of both PC1/3 and PC2 in the lysates and had no apparent effect on the contaminating activities. In addition, substitution of an Asn for Leu in the reactive site of STI-2 obliterated all inhibitory activity, as shown in Figures 9A - 9C.

Figures 9D - 9F demonstrate that a related molecule, SIL-2, is also a partial inhibitor of PC2, but that the inhibition becomes complete if the preparation is first subjected to size fractionation. This result demonstrates that the contaminating activities are smaller than about 30,000 daltons and that it should be possible to easily purify the PC2 isozyme.

The SIL-2 peptide, as illustrated in Figures 9 E and 9F, and the API-2C peptide exhibited moderate inhibitory activity toward PC2 but were ineffective against PC1/3 in either the fluorometric or the proglucagon conversion assays.

These results demonstrate that peptides or full length SSI-like inhibitors that contain a basic amino acid in their reactive site are suitable as PC inhibitors. Having identified proteins or small peptides that inhibit the PCs, a mammalian

expression vector(s) carrying an insert that coded for a product that would be vectorially transported into the regulated secretory pathway and which would also contain the inhibitor sequence can be developed. If the inhibitor sequence were to be co-packaged with the PCs and their substrates in secretory granules, the likelihood of PC inhibition is greatly increased.

Oligonucleotides that code for all or part of the sequences determined to be effective in PC inhibition in the *in vitro* studies are linked downstream of the coding region of a preprohormone. For the purpose of the present example only, the prepro- region of preprosomatostatin is chosen. The primary structure of this precursor is depicted below with the peptide hormone cleavage sites shown in bold type and underlined.

H_2N-MLSCRLQCALAALSIVLALGCVTGAPSDPRLRQFLQKSLAAAAGKQELAKYFLAELLSEPN

Signal Peptide

QTENDALEPEDLSQAA EQDEMRLELQRSANSNPAMAPRERKAGCKNFFWKTFTSC-COOH

Δ

Termination point after PvuII cleavage of cDNA at base 329

Somatostatin-14

Somatostatin-28

This sequence is particularly suitable as the "carrier" to deliver inhibitor sequences to the regulated secretory pathway for several reasons. First, as far as is known, the "pro- region" of this precursor is not normally processed in mammalian cells, and it does not have any demonstrated biologic activity. Second, the "pro-region" of prosomatostatin is known to target the precursor into the regulated secretory pathway, ultimately to be stored and processed in secretory granules. Third, the portion of the precursor that contains somatostatin-28 (SS-28) and somatostatin-14 (SS-14), the bioactive products of this precursor, can be easily eliminated from our proposed "carrier" by restriction digestion of the cDNA. Dual digestion of the rat preprosomatostatin cDNA with XbaI and PvuII will cut the plasmid at base positions 53 and 329, respectively. In this cDNA, base 53 is located 58 bases upstream from the ATG start codon, and base 329 occurs 32 bases upstream from the codon that

designates the single Arg residue that serves as the cleavage site for releasing SS-28 from prosomatostatin. Use of the endonuclease cleavage site at base 329 is particularly important because it will eliminate the coding region for both of the bioactive products (SS-14 and SS-28) and their respective cleavage sites from the prepro- region that is intended to serve as the carrier for vectorial transport into the endoplasmic reticulum. Once the "carrier" coding DNA is available, then oligonucleotides that have been prepared to code for the specific inhibitors are ligated to the 3' end of the prepro- region of the "carrier". The 3' end of these oligos will contain a ClaI site to accommodate directional insertion into the vector. This chimeric coding region is then ligated into the modified pOPRSV eukaryotic expression vector which has been modified to allow directional insertion of inserts with a 5' XbaI and a 3' ClaI site. After transfection into αTC1-6 cells, the insert then codes for a chimeric peptide in the transfected cells. Figure 12 depicts representative examples of several chimeric peptides of this type.

Figure 12 provides a schematic of the "carrier" preprosomatostatin (rectangles) and the peptide products of the chimeric cDNA constructs coding for amino acid sequences representing PC inhibitors. These latter sequences replace the C-terminal somatostatin-14 and somatostatin-28 peptides normally expressed as part of preprosomatostatin. The inhibitors shown are: (A) STI-2 peptide, (B) the SIL-2 peptide, (C) API-2C peptide, (D) the SIL-4 peptide. The reactive site residues of the subtilisin inhibitors are underlined.

The spatial arrangement of these chimeric peptides places the potential cleavage site (or inhibitor reactive site) in approximately the same position relative to the initiator Met in preprosomatostatin as the single Arg that serves as the cleavage site for somatostatin-28 in the native precursor.

The LacSwitch eukaryotic expression system (Stratagene) consists of a eukaryotic Lac-repressor expressing vector (p3'SS) and two eukaryotic lac-operator containing vectors into one of which the cDNA of interest is inserted by cloning. Normally both LacSwitch vectors are transfected into a cultured cell line where

expression of the coding region inserted into pOPRSVCAT is repressed by the product of the second vector until an inducer (for example IPTG) is added to the media. Upon induction, expression of the sequence inserted into pOPRSVCAT resumes. However, the pOPRSV plasmid can also be transfected alone, allowing it to constitutively express the product(s) coded for by the insert it carries.

Because constitutive expression of the vector driven products is sought, transfections will be performed using the pOPRSV vector only. A modified form of the pOPRSV vector is used for introduction of inserts. The modified vector was constructed by ligating the multiple cloning site (MCS) of pBluescript KS+ with that of the MCS of pREP9 at the Kpnl site. The new construct is then digested with Notl and ligated into the Notl sites of the pOPRSV vector, replacing the chloramphenicol acetyl transferase (CAT) reporter gene. The modified (pOPRSVmod) vector is then transformed into DH5αF cells (Gibco/BRL). The modified vector allows for directional incorporation of inserts in either the antisense or sense orientation using the ClaI and XbaI restriction enzyme sites.

Cells to be transfected with the pOPRSV vector + chimeric inhibitor inserts are plated in triplicate into 60 mm culture dishes and cultured in normal growth media overnight prior to transfection. On the day of the transfections, cells in the dishes are washed twice with prewarmed Hanks Balanced Salt Solution (HBSS) prior to the addition of transfection media (the approximate cell density in each dish will not exceed 2-4 x 10^3). The transfection media consists of 20 µg of plasmid and 60 µg of Lipofectamine (Gibco/BRL) in 1.5 ml DMEM with no antibiotics or serum. The protocol for these transfections is adapted from the Gibco/BRL transfection protocol. The DNA-Lipofectamine solution incubates at room temperature for 45 min prior to addition to cells. The cells are then incubated in the transfection media for 6 hours at 37°C under 5% CO_2, after which normal growth medium is added and the cells incubated overnight. The transfection efficiency of this protocol was determined to be approximately 84% using the pCH110 β-galactose expression vector (Pharmacia Biotech). Important controls include "transfection" incubations of cells with

transfection media without plasmid, plasmids without inserts, and cells which were not treated. One day after performing the transfections, incubation in media containing G418 is used to achieve selection of transfected cells to identify stable transfectants.

Cells from parallel sets of dishes are used for assessing endogenous processing activity. This involves glucagon RIA of media samples, metabolic labeling, extraction, HPLC separations and peptide mapping analyses to determine proglucagon product/precursor ratios. Processing in transfected cells can be initially assessed by glucagon RIA of aliquots from media samples. The glucagon RIA can be based on the 04A antiserum. The RIA based on this antiserum detects only the free C-terminus of glucagon and is thus not reactive to proglucagon or any cleavage intermediate of the precursor. Initially, media samples are taken for RIA starting at day 1 after transfection to determine the optimal culture time after transfection for assessing the effects of initiator expression. Glucagon levels secreted from cells transfected with vector only and cells transfected with insert containing vector are compared. When reductions of glucagon release are observed, cells are taken for metabolic labeling and product analyses.

Several additional control experiments can also be performed. Immunohistochemistry to localize the N-terminal pro- region of the chimeric carrier domain is performed to demonstrate targeting of the chimeric proteins into the regulated secretory pathway. An antiserum directed toward an epitope near the N-terminus of prosomatostatin can be used for this purpose. To determine if the products from the chimeric inserts are co-localized with the PCs in secretory granules, αTC1-6 cells are plated in Lab-Tek® Chamber slides (Nunc) and transfected as previously described. After the allotted incubation period, the cells are fixed and stained utilizing the N-terminally directed prosomatostatin primary antibody and an anti-rabbit-FITC secondary antibody. If necessary, electron microscopy can be utilized to define the exact localization of the inhibitor construct in the secretory pathway of the αTC1-6 cells. Also, each day one set of dishes can be removed from culture for microscopic determination of cell morphology and cell viabilities with Trypan Blue.

For expression of any of the chimeric inserts to be successful, it is essential to assure that ligation of the coding region of interest to the prepro- carrier does not introduce shifts in reading frame. If appropriate restriction sites cannot be identified, synthetic oligonucleotide linkers can be prepared and ligated to the 3' end of the carrier prior to linking SSI-like coding regions to assure in frame reading of the cDNA. DNA sequencing is performed to verify that the inserts employed will code appropriately for the protein inhibitor of interest.

It is possible that preprosomatostatin that does not exhibit its normal cleavage sites for processing somatostatin-28 and somatostatin-14 from the precursor could also serve as an effective inhibitor of the PCs in intact cells. Both PC1/3 and PC2 can cleave at the RK site in prosomatostatin to release SS-14. A non-PC aspartyl protease and PC1/3 can release SS-28 from prosomatostatin. Accordingly, if both of the susceptible cleavage sites in prosomatostatin were altered, and the resulting mutant precursor were introduced into the regulated secretory pathway of cells making bioactive peptides, it could compete with the natural substrate for the PCs and thereby inhibit PC activity. As an alternative approach to use of the chimeric inhibitors described above, we plan to test the efficacy of mutagenized prosomatostatin as a PC inhibitor in αTC1-6 cells. Using the protocols and reagents available in either the Chameleon Double Stranded Site Directed Mutagenesis kit from Stratagene, or the Morph Kit from 5 Prime - 3 Prime, we will alter the coding region of the rat preprosomatostatin cDNA to introduce substitutions at the single Arg (SS-28) and the Arg-Lys (SS-14) cleavage sites. By introducing either Met or Leu at the single Arg site and at the Lys site of the basic pair, PC mediated cleavage at these positions should be prevented. After preparation of the mutant preprosomatostatin cDNA the complete coding sequence will be recovered from its parent vector (pRTB143) using dual digestion with XbaI and ClaI. After gel purification of the resulting fragment, both the 5' and 3' ends will be blunt ended using the Klenow fragment of DNA polymerase I. This DNA fragment will be ligated into the pOPRSVmod expression vector predigested with XbaI and ClaI and blunt ended. The vector carrying this insert

will then be transformed into competent cells for amplification to verify insertion and determine correct orientation by either restriction digestion or sequencing. Clones containing the insert in the correct orientation are used for transfection into αTC1-6 cells as discussed previously. To determine whether the modified precursor is being targeted to secretory granules, immunocytochemistry may be employed using the N-terminally directed prosomatostatin antiserum as the primary antiserum. Transfections of vectors carrying either non-mutated preprosomatostatin cDNA inserts or no insert will be employed as controls. If sufficiently high levels of the modified prosomatostatin are expressed in these cells and targeted into the regulated secretory pathway, proglucagon processing would likely be impaired. This impairment could be exhibited initially by reduction of glucagon secreted into culture media of transfected cells as determined by glucagon RIA. If glucagon levels are reduced, then inhibition of PC mediated processing at specific cleavage sites will be assayed using the established metabolic labeling and HPLC product analysis protocol.

Experiments are undertaken to identify peptide PC inhibitors of HIV. Recombinant furin, PC6A and PC7 are incubated with a fluorogenic substrate ± the following synthetic peptides:

RGDVACTKQFDPVVVT (SEQ ID NO: 1),
GEDVMCPMVYDPVLLT (SEQ ID NO: 2),
DTGRVCTREYRPVTVS (SEQ ID NO: 3),
RDGVICNKLYDPVVVT (SEQ ID NO: 4),
ADGVMCTREYAPVVVT (SEQ ID NO: 5),
SEGVMCPMIYDPVLLT (SEQ ID NO: 6),
CALEGSLQKRGIVEQCC (SEQ ID NO: 7),
CALEGSLQKHGIVEQCC (SEQ ID NO: 8),
CRDGVICNKLYDPVVVTC (SEQ ID NO: 9),
CADGVMCTREYAPVVVTC (SEQ ID NO: 10),
CDTGRVCTREYRPVTVSC (SEQ ID NO: 11),
CRGDVACTKQFDPVVVTC (SEQ ID NO: 13),

CGEDVMCPMVYDPVLLTC (SEQ ID NO: 14),
CSEGVMCPMIYDPVLLTC (SEQ ID NO: 15),
RDGVICNKNYDPVVVT (SEQ ID NO: 16),
DDAVMCTREYAPVVVT (SEQ ID NO: 17),
DQDRACIKIYDPLVVT (SEQ ID NO: 18),
DAGVMCTREYAPVVVT (SEQ ID NO: 19),
RDGVMCTKQYDPVVVT (SEQ ID NO: 20),
EPGRMCTKEWRPITVT (SEQ ID NO: 21),
RDDVWCNKLYDPVVVT (SEQ ID NO: 22),
EDSVMCTREYAPVVVT (SEQ ID NO: 23), or
TGDVMCTKQYDVVVT (SEQ ID NO: 24).

This fluorometric assay may also be adapted to testing the efficacy of inhibitors of HIV gp 160 surface envelope protein processing. Peptide and full length inhibitors shown to be effective in the fluorometric assay are then tested for their ability to inhibit cleavage of the target substrate, gp160. The gp160 cleavage assay is based on similar techniques known in the art. See E. Decroley et al. (1994) *J. Biol. Chem.* 269: 12240-12247 and L. Miranda et al. (1996) *Proc. Natl. Acad. Sci. USA* 93: 7695-7700. Commercial gp160 may be used, although immunoreactive epitopes could be differentially mapped on western blots, leading to inconsistent quantitation. Therefore, gp160 is preferably prepared by infecting CV-1 cells with a VV:gp160 construct at a multiplicity of infection of 1-5 pfu/cell. After an 18-20 hours incubation, cells are placed in Met and Cys-depleted DMEM for 1 hour, and then cultured for 6 hours in the same medium supplemented with 150 μCi ^{35}S-Met/^{35}S-Cys. Cells are then lysed in 30mM Tris-HCL, pH 7.4, 150 mM NaCl, 1% Triton X-100, 0.5% sodium deoxycholate. After centrifugation, the ^{35}S-labeled gp160 is separated from the soluble components using a lentil lectin agarose column, and then eluted in lysis buffer containing 0.5M α-methylmannoside.

The ^{35}S-labeled gp160 (10,000 cpm) is then incubated overnight with either furin, PC6A or PC7 in 50mM Tris/acetate supplemented with 1% Triton Z-100 and 2mM calcium acetate. The pH is adjusted as indicated for the previous fluorometric assay. Incubates are then subjected to SDS PAGE on 8% gels and quantification of gp160 and its cleavage products is analyzed by a phosphoimager. Enhanced sensitivity can be achieved by preparing fluorograms, scanning to generate digital images, and using the NIH IMAGE program to quantitate band intensity. Each inhibitor is incubated with the PC for 30 minutes prior to the addition of labeled gp160. The results determine whether an inhibitor can successfully compete with gp160 to inhibit PC activity.

Any peptide shown to be an inhibitor in the fluorometric assay or gp160 conversion assays is then tested to determine the likely mechanisms of enzyme action. First, the question of whether inhibitor proteins are cleaved by PCs is determined by HPLC. Incubates of the recombinant PC plus full length inhibitors are subjected to reverse-phase HPLC, which permits differentiation of cleavage products from the intact protein. For example, the STI-2 full length protein has a calculated value of 339, whereas the N-terminal fragment after cleavage is 290 and the C terminal fragment is 46. Therefore, cleavage products would be expected to elute earlier than the intact protein on HPLC. Peptide incubates are similarly analyzed by HPLC. If necessary, inhibitors may be iodinated to determine whether the inhibitor binds the enzyme tightly and is only cleaved very slowly. For example, STI-2 has 4 tyrosines, which would be distributed so that each of the potential cleavage products indicated above would be labeled. The HPLC is then repeated with iodinated inhibitors to determine whether small amounts of cleavage products are released slowly. If the results suggest that the inhibitors are not cleaved by the PCs, it is assumed that they are bound to the enzymes but not cleaved. In either case, IC_{50} and V/Km values will be determined for the peptides and full length inhibitors. Second, the question of whether inhibition is highly sequence dependent is addressed using sequence modified peptides and full length inhibitors. The results of these two tests provide information

useful in identifying the inhibitors that have potential to function most effectively *in vivo*.

An *in vivo* assay is performed to identify peptides and full length proteins that inhibit PC processing in HIV target cells. Any peptide or full length sequence identified as an inhibitor in both the fluorometric and gp160 conversion assay is tested *in vivo*. A mammalian expression vector is prepared for a peptide according to the previously presented protocol. Thus, a chimeric cDNA construct is created comprising a nucleic acid coding sequence of the peptide(s) of interest linked downstream of the nucleic acid coding region of a carrier peptide. The construct may comprise one or more nucleic acid coding sequences, encoding one or more peptides of interest. The carrier peptide is preferably the prepro-region of a preprohormone, such as prosomatostatin. After insertion into the eukaryotic expression vector, the chimeric insert will then code for a chimeric peptide in transfected cells.

Alternatively, transfection vectors for full length inhibitors are prepared by ligating coding inserts into the multiple cloning site of the pSecTag2 mammalian expression vector (Invitrogen). This vector incorporates a coding region for a signal peptide shown to be effective in targeting nascent polypeptide chains into the secretory pathway of mammalian cells. This vector is provided in three forms, with two being extended in the multiple cloning site by one or two nucleotides, respectively, to facilitate incorporation of the insert for in frame reading. Oligonucleotide priming of coding sequences is used for DNA sequence analysis to verify that the sequences are correctly oriented in the vector. The pSecTag 2 vector is useful for other reasons as well. Specifically, the vector contains six histidine residues which facilitate purification using a nickel resin column. This procedure can be used to verify synthesis of the inhibitor protein in transfected cells. Moreover, an antibody to C-myc can be used to monitor transfection efficiencies and expression levels when an antibody to the inhibitor is unavailable.

An alternative approach is to transfect the preprosomatostatin or inhibitor chimeras into HIV taget cells. Complete, or nearly complete, inhibition of PC

cleave of gp 160 may depend upon the level of expression of the inhibitor protein. It is desirable to achieve as high expression of inhibitor protein as is feasible. For example, inhibitor to PC ratios of 5:1 are desirable. Inhibitor expression may be enhanced by using an inducible vector. Where vectors are used therapeutically, the TAT responsive element of the 5'LTR of HIV may be useful as an inducible element. The 5'LTR of HIV-1 is placed upstream of the coding sequences for either full-length SSI-like proteins or prepro-peptide/chimeric constructs yielding SSI reactive site sequences. There it can serve as a promoter, but expression of inhibitor remains latent until the host cell is infected with HIV.

Levels of inhibitor may also be enhanced by using a vector comprising a Golgi retention signal. See, e.g., Pfeiffer, T. et al.(1997) *J. Gen. Virol.* 78: 1745-1753. This signal prevents a foreign protein like the inhibitor from being directed to the lysosomes for degradation. Incorporation of a Golgi retention signal into the inhibitor vector would ensure that inhibitor protein is retained in the Golgi.

The efficacy of the inhibitors is examined in both CD4+ lymphoid and monocyte/macrophage cells. CD4+ cell lines may include Jurkat, SupT_1, T_1, or T_2 lymphoblasts. Monocyte/macrophage cell lines may include U937 and THP-1. Either provides an ideal model cell system, as each permits the study of inhibitors in HIV target cells. Cells are cultured under recommended conditions in 25 cm^2 dishes, and then transfected with vectors containing inhibitor coding inserts. Cells are then transfected with VV:gp160 vectors, at a multiplicity of infection of 1 to 5 pfu/cell. Control transfections are performed identically, except that the control vectors contain no inhibitor inserts. Transfection efficiencies are monitored immunologically using C-myc antisera (for pSecTag2 vectors) or Pro-SS peptide antisera, either in cultured cells or by western analysis of cell extracts. Transfection efficiencies can be increased as needed, by electroporation or other known methods. If vaccinia infection predominates in any of the cells used, VV:gp160 will be replaced by PCRCMV-gp160 (Invitrogen).

Transfected cells are then washed and preincubated for 1 hour in Cys and Met-depleted media. Cells are then incubated for 4 hours in media supplemented with ^{35}S-Cys and ^{35}S-Met. Media is recovered and centrifuged for 10 minutes at 300g to remove cells and debris. An equal volume of an inhibitor cocktail containing 1mM EDTA, 2μg/ml aprotinin, and 100μg/ml PMSF is added to prevent proteolytic degradation of labeled products secreted into the media. Cells remaining in the culture dishes and those recovered by centrifugation are washed once in PBS and then extracted with a lysis buffer (50mM Tris-HCl, pH 7.5, 1% Triton X-100, 0.5% deoxycholate, 150 mM NaCl and 1% SDS). Media and lysate are precleared by adding an equal volume of 50% (w/v) protein A-Sepharose beads and rocking for 4 hours at 4°C. The beads are removed by centrifugation, and the supernatants placed in separate tubes to which an appropriate amount of gp160/120/41 antisera is added. New protein A-Sepharose beads are added and rocked for an additional 4 hours at 4°C. Then the beads are washed three times with lysis buffer and removed by centrifugation. The antibody-bound components are released by incubation in SDS PAGE sample buffer. Samples are then concentrated and subjected to PAGE on 8% gels. Quantification of gp160 and its cleavage products is then performed.

Two additional assays are performed to assess inhibitor efficacy: First, a cell fusion assay is performed to monitor the extent to which gp120 is displayed on the surface of cells expressing inhibitors. See C. Yang et al. (1996) *Virology* 221: 87-97. Cells are seeded into six-well plates one day prior to infection with VV:gp160. One day after VV:gp160 infection, the infected cells are overlaid with CD4+ cells. Cell fusion is monitored and photographed 4-8 hours later by phase contrast microscopy. A reduction in syncytia formation in cells expressing inhibitors relative to those cells transfected with a control vector indicates diminished gp120 expression. Alternatively, fusion can be monitored in cells transfected with the plasmid G1NT7β-gal or infected with vTF7-3 by a colorimetric assay.

Second, a biotinylation assay is conducted to determine cell surface gp120 expression. After transfection and radiolabeling, cells are biotinylated in PBS

with 0.5 mg/ml of sulfo-succinimidyl-2(biotinamido)ethyl-1,3-dithiopropionate (NHS-SS-biotin, Pierce) for 30 minutes at 4°C, followed by lysis in lysis buffer. Lysates are then immunoprecipated with the appropriate antisera/ Protein-A Agarose overnight at 4°C. Samples are washed twice in lysis buffer plus 0.4% SDS and heated at 95°C for 15 minutes. Dissociated proteins are dissolved in lysis buffer and incubated with Streptavidin-agarose (Pierce) for 5 hours at 4°C. Biotinylated samples are then washed three times with lysis buffer, combined with reducing sample buffer, heated and loaded onto SDS gels. The gp160 protein and its cleavage products in media, cell lysates, and at the cell surface will be quantitated as previously described in C. Spies et al. *J. Virol.* 68:585-591, incorporated herein by reference. A reduction in surface expression of gp120 indicates a finding of inhibitor efficacy.

Experiments are then performed using animal models. Any peptide or full length inhibitor shown to inhibit PC processing of gp 160 in HIV target cells is tested in an animals model. CD4+ and/or CD8+ lymphocyte precursors or monocyte/macrophage precursors are isolated from mice, rats or non-human primates. These cells are transfected *in vitro* with expression vectors comprising the inhibitor of interest, according to Apperly, J.F., et al. (1991) *Blood* 78: 310-317; Bunnell, B.A. et al. (1995) *Proc. Natl. Acad. Sci. USA* 92: 7739-7743; Morgan, R.A. (1996) Gene Therapy. In Immunology of HIV Infection. S. Gupta, editor. Plenum Press New York. 577-594. After amplification of transfected cells, they are retransfused into host animal(s). Test animals are then infected with HIV, and viral load is monitored over time using established procedures. A reduction in viral load in an animal transfused with transfected cells relative to viral load in mock transfected animals indicates the viability of this methodology as a treatment for HIV.

Obviously, numerous modifications and variations of the present invention are possible in light of the above teachings. It is therefore to be understood that within the scope of then appended claims, the invention may be practiced otherwise than as specifically described herein.

SEQUENCE LISTING

(1) GENERAL INFORMATION:

(i) APPLICANT: Noe, Bryan D.
Rothenberg, Mark E.

(ii) TITLE OF INVENTION: PEPTIDE INHIBITORS OF PROPEPTIDE/PROHORMONE CONVERTASES

(iii) NUMBER OF SEQUENCES: 50

(iv) CORRESPONDENCE ADDRESS:
(A) ADDRESSEE: KILPATRICK STOCKTON LLP
(B) STREET: Suite 2800, 1100 Peachtree Street, NE
(C) CITY: Atlanta
(D) STATE: Georgia
(E) COUNTRY: USA
(F) ZIP: 30309-4530

(v) COMPUTER READABLE FORM:
(A) MEDIUM TYPE: Floppy disk
(B) COMPUTER: IBM PC compatible
(C) OPERATING SYSTEM: PC-DOS/MS-DOS
(D) SOFTWARE: PatentIn Release #1.0, Version #1.25

(vi) CURRENT APPLICATION DATA:
(A) APPLICATION NUMBER: PCT/US98/
(B) FILING DATE: 25 February 1998
(C) CLASSIFICATION:

(vii) PRIOR APPLICATION DATA:
(A) APPLICATION NUMBER: 60/039,129
(B) FILING DATE: 02 March 1997

(viii) ATTORNEY/AGENT INFORMATION:
(A) NAME: Meredith Esq., Roy D.
(B) REGISTRATION NUMBER: 30,777
(C) REFERENCE/DOCKET NUMBER: EMU155PCT

(ix) TELECOMMUNICATION INFORMATION:
(A) TELEPHONE: 404.815.6500
(B) TELEFAX: 404.815.6555

(2) INFORMATION FOR SEQ ID NO:1:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:1:

Arg Gly Asp Val Ala Cys Thr Lys Gln Phe Asp Pro Val Val Val Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:2:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:2:

Gly Glu Asp Val Met Cys Pro Met Val Tyr Asp Pro Val Leu Leu Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:3:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:3:

Asp Thr Gly Arg Val Cys Thr Arg Glu Tyr Arg Pro Val Thr Val Ser
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:4:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:4:

Arg Asp Gly Val Ile Cys Asn Lys Leu Tyr Asp Pro Val Val Val Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:5:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:5:

Ala Asp Gly Val Met Cys Thr Arg Glu Tyr Ala Pro Val Val Val Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:6:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:6:

Ser Glu Gly Val Met Cys Pro Met Ile Tyr Asp Pro Val Leu Leu Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:7:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:7:

Cys Ala Leu Glu Gly Ser Leu Gln Lys Arg Gly Ile Val Glu Gln Cys
1 5 10 15

Cys

(2) INFORMATION FOR SEQ ID NO:8:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 17 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:8:

Cys Ala Leu Glu Gly Ser Leu Gln Lys His Gly Ile Val Glu Gln Cys
1 5 10 15

Cys

(2) INFORMATION FOR SEQ ID NO:9:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:9:

Cys Arg Asp Gly Val Ile Cys Asn Lys Leu Tyr Asp Pro Val Val Val
1 5 10 15

Thr Cys

(2) INFORMATION FOR SEQ ID NO:10:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:10:

Cys Ala Asp Gly Val Met Cys Thr Arg Glu Tyr Ala Pro Val Val Val
1 5 10 15

Thr Cys

(2) INFORMATION FOR SEQ ID NO:11:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:11:

Cys Asp Thr Gly Arg Val Cys Thr Arg Glu Tyr Arg Pro Val Thr Val
1 5 10 15

Ser Cys

(2) INFORMATION FOR SEQ ID NO:12:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:12:

Cys Arg Asp Gly Val Ile Cys Asn Lys Leu Thr Asp Pro Val Val Val
1 5 10 15

Thr Cys

(2) INFORMATION FOR SEQ ID NO:13:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:13:

Cys Arg Gly Asp Val Ala Cys Thr Lys Gln Phe Asp Pro Val Val Val
1 5 10 15

Thr Cys

(2) INFORMATION FOR SEQ ID NO:14:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:14:

Cys Gly Glu Asp Val Met Cys Pro Met Val Tyr Asp Pro Val Leu Leu
1 5 10 15

Thr Cys

(2) INFORMATION FOR SEQ ID NO:15:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 18 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:15:

Cys Ser Glu Gly Val Met Cys Pro Met Ile Tyr Asp Pro Val Leu Leu
1 5 10 15

Thr Cys

(2) INFORMATION FOR SEQ ID NO:16:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:16:

Arg Asp Gly Val Ile Cys Asn Lys Asn Tyr Asp Pro Val Val Val Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:17:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:17:

Asp Asp Ala Val Met Cys Thr Arg Glu Tyr Ala Pro Val Val Val Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:18:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:18:

Asp Gln Asp Arg Ala Cys Ile Lys Ile Tyr Asp Pro Leu Val Val Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:19:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:19:

Asp Ala Gly Val Met Cys Thr Arg Glu Tyr Ala Pro Val Val Val Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:20:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:20:

Arg Asp Gly Val Met Cys Thr Lys Gln Tyr Asp Pro Val Val Val Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:21:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:21:

Glu Pro Gly Arg Met Cys Thr Lys Glu Trp Arg Pro Ile Thr Val Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:22:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:22:

Arg Asp Asp Val Trp Cys Asn Lys Leu Tyr Asp Pro Val Val Val Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:23:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:23:

Glu Asp Ser Val Met Cys Thr Arg Glu Tyr Ala Pro Val Val Val Thr
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:24:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 15 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:24:

```
Thr Gly Asp Val Met Cys Thr Lys Gln Tyr Asp Val Val Val Thr
1               5                  10                  15
```

(2) INFORMATION FOR SEQ ID NO:25:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 110 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:25:

```
Ala Ser Leu Tyr Ala Pro Ser Ala Leu Val Leu Thr Val Gly His Gly
1               5                  10                  15

Thr Ser Ala Ala Ala Ala Ser Pro Leu Arg Ala Val Thr Leu Asn Cys
            20                  25                  30

Ala Pro Thr Ala Ser Gly Thr His Pro Ala Pro Ala Leu Ala Cys Ala
        35                  40                  45

Asp Leu Arg Gly Val Gly Gly Asp Ile Asp Ala Leu Lys Ala Arg Asp
    50                  55                  60

Gly Val Ile Cys Asn Lys Leu Tyr Asp Pro Val Val Val Thr Val Asp
65                  70                  75                  80

Gly Val Trp Gln Gly Lys Arg Val Ser Tyr Glu Arg Thr Phe Gly Asn
                85                  90                  95

Glu Cys Val Lys Asn Ser Tyr Gly Thr Ser Leu Phe Ala Phe
            100                 105                 110
```

(2) INFORMATION FOR SEQ ID NO:26:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 110 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:26:

Ala Ser Leu Tyr Ala Pro Ser Ala Leu Val Leu Thr Val Gly His Gly
1 5 10 15

Thr Ser Ala Ala Ala Ala Ser Pro Leu Arg Ala Val Thr Leu Asn Cys
20 25 30

Ala Pro Thr Ala Ser Gly Thr His Pro Ala Pro Ala Leu Ala Cys Ala
35 40 45

Asp Leu Arg Gly Val Gly Gly Asp Ile Asp Ala Leu Lys Ala Arg Asp
50 55 60

Gly Val Ile Cys Lys Lys Leu Tyr Asp Pro Val Val Val Thr Val Asp
65 70 75 80

Gly Val Trp Gln Gly Lys Arg Val Ser Tyr Glu Arg Thr Phe Gly Asn
85 90 95

Glu Cys Val Lys Asn Ser Tyr Gly Thr Ser Leu Phe Ala Phe
100 105 110

(2) INFORMATION FOR SEQ ID NO:27:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 110 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:27:

Ala Ser Leu Tyr Ala Pro Ser Ala Leu Val Leu Thr Val Gly His Gly
1 5 10 15

Thr Ser Ala Ala Ala Ala Ser Pro Leu Arg Ala Val Thr Leu Asn Cys
20 25 30

Ala Pro Thr Ala Ser Gly Thr His Pro Ala Pro Ala Leu Ala Cys Ala
35 40 45

Asp Leu Arg Gly Val Gly Gly Asp Ile Asp Ala Leu Lys Ala Arg Asp
50 55 60

Gly Val Ile Cys Arg Lys Leu Tyr Asp Pro Val Val Val Thr Val Asp
65 70 75 80

Gly Val Trp Gln Gly Lys Arg Val Ser Tyr Glu Arg Thr Phe Gly Asn
85 90 95

Glu Cys Val Lys Asn Ser Tyr Gly Thr Ser Leu Phe Ala Phe

100 105 110

(2) INFORMATION FOR SEQ ID NO:28:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:28:

Ser Leu Tyr Ala Pro Ser Ala Leu Val Leu Thr Val Gly His Gly Glu
1 5 10 15

Ser Ala Ala Thr Ala Ala Pro Leu Arg Ala Val Thr Leu Thr Cys Ala
20 25 30

Pro Thr Ala Ser Gly Thr His Pro Ala Ala Ala Ala Ala Cys Ala Glu
35 40 45

Leu Arg Ala Ala His Gly Asp Pro Ser Ala Leu Ala Ala Glu Asp Ser
50 55 60

Val Met Cys Thr Arg Glu Tyr Ala Pro Val Val Val Thr Val Asp Gly
65 70 75 80

Val Trp Gln Gly Arg Arg Leu Ser Tyr Glu Arg Thr Phe Ala Asn Glu
85 90 95

Cys Val Lys Asn Ala Gly Ser Ala Ser Val Phe Thr Phe
100 105

(2) INFORMATION FOR SEQ ID NO:29:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 113 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:29:

Thr Ala Pro Ala Ser Leu Tyr Ala Pro Ser Ala Leu Val Leu Thr Ile
1 5 10 15

Gly Gln Gly Glu Ser Ala Ala Ala Thr Ser Pro Leu Arg Ala Val Thr
20 25 30

Leu Thr Cys Ala Pro Lys Ala Thr Gly Thr His Pro Ala Ala Asp Ala
35 40 45

Ala Cys Ala Glu Leu Arg Arg Ala Gly Gly Asp Phe Asp Ala Leu Ser
50 55 60

Ala Ala Asp Gly Val Met Cys Thr Arg Glu Tyr Ala Pro Val Val Val
65 70 75 80

Thr Val Asp Gly Val Trp Gln Gly Arg Arg Leu Ser Tyr Glu Arg Thr
85 90 95

Phe Ala Asn Glu Cys Val Lys Asn Ala Gly Ser Ala Ser Val Phe Thr
100 105 110

Phe

(2) INFORMATION FOR SEQ ID NO:30:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 110 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:30:

Ser Leu Tyr Ala Pro Ser Ala Val Val Ile Ser Lys Thr Gln Gly Ala
1 5 10 15

Ser Ala Asp Ala Pro Ala Gln Arg Ala Val Thr Leu Arg Cys Leu Pro
20 25 30

Val Gly Gly Asp His Pro Ala Pro Glu Lys Ala Cys Ala Ala Leu Arg
35 40 45

Glu Ala Gly Gly Asp Pro Ala Ala Leu Pro Arg Tyr Val Glu Asp Thr
50 55 60

Gly Arg Val Cys Thr Arg Glu Tyr Arg Pro Val Thr Val Ser Val Gln
65 70 75 80

Gly Val Trp Asp Gly Arg Arg Ile Asp His Ala Gln Thr Phe Ser Asn
85 90 95

Ser Cys Glu Leu Glu Lys Gln Thr Ala Ser Val Tyr Ala Phe
100 105 110

(2) INFORMATION FOR SEQ ID NO:31:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:31:

Tyr Ala Pro Ser Ala Leu Val Leu Thr Val Gly His Gly Glu Ser Ala
1 5 10 15

Ala Thr Ala Ala Pro Leu Arg Ala Val Thr Leu Thr Cys Ala Pro Thr
20 25 30

Ala Ser Gly Thr His Pro Ala Ala Asp Ala Ala Cys Ala Glu Leu Arg
35 40 45

Ala Ala His Gly Asp Pro Ser Ala Leu Ala Ala Asp Asp Ala Val Met
50 55 60

Cys Thr Arg Glu Tyr Ala Pro Val Val Val Thr Val Asp Gly Val Trp
65 70 75 80

Gln Gly Arg Arg Leu Ser Tyr Glu Arg Thr Phe Ala Asn Glu Cys Val
85 90 95

Lys Asn Ala Gly Ser Ala Ser Val Phe Thr Phe
100 105

(2) INFORMATION FOR SEQ ID NO:32:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 116 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:32:

Ala Pro Asp Ala Ala Pro Ala Ser Leu Tyr Ala Pro Ser Ala Leu Val
1 5 10 15

Leu Thr Ile Gly His Gly Gly Ala Ala Ala Thr Ala Thr Pro Glu Arg
20 25 30

Ala Val Thr Leu Thr Cys Ala Pro Thr Ser Ser Gly Thr His Pro Ala
35 40 45

Ala Ser Ala Ala Cys Ala Glu Leu Arg Gly Val Gly Gly Asp Phe Ala

50 55 60

Ala Leu Lys Ala Arg Asp Asp Val Trp Cys Asn Lys Leu Tyr Asp Pro
65 70 75 80

Val Val Val Thr Ala Gln Gly Val Trp Gln Gly Gln Arg Val Ser Tyr
85 90 95

Glu Arg Thr Phe Gly Asn Ser Cys Glu Arg Asp Ala Val Gly Gly Ser
100 105 110

Leu Phe Ala Phe
115

(2) INFORMATION FOR SEQ ID NO:33:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 111 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:33:

Ser Leu Tyr Ala Pro Ser Ala Met Val Phe Ser Val Ala Gln Gly Asp
1 5 10 15

Asp Val Ala Ala Pro Thr Val Val Arg Ala Thr Thr Val Ser Cys Ala
20 25 30

Pro Gly Ala Arg Gly Thr His Pro Asp Pro Lys Ala Ala Cys Ala Ala
35 40 45

Leu Lys Ser Thr Gly Gly Ala Phe Asp Arg Leu Leu Ser Glu Pro Asn
50 55 60

Pro Asp Arg Ala Cys Pro Met His Tyr Ala Pro Val Thr Val Ser Ala
65 70 75 80

Val Gly Val Trp Glu Gly Arg Arg Val Ala Trp Asp His Thr Phe Ala
85 90 95

Asn Ser Cys Thr Met Ala Ala Thr Leu Asp Gly Asn Ala Val Phe
100 105 110

(2) INFORMATION FOR SEQ ID NO:34:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single

(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:34:

Gly Leu Tyr Ala Pro Ser Ala Leu Val Leu Thr Met Gly His Gly Asn
1 5 10 15

Ser Ala Ala Thr Val Asn Pro Glu Arg Ala Val Thr Leu Asn Cys Ala
20 25 30

Pro Thr Ala Ser Gly Thr His Pro Ala Ala Leu Gln Ala Cys Ala Glu
35 40 45

Leu Arg Gly Ala Gly Gly Asp Phe Asp Ala Leu Thr Val Arg Gly Asp
50 55 60

Val Ala Cys Thr Lys Gln Phe Asp Pro Val Val Val Thr Val Asp Gly
65 70 75 80

Val Trp Gln Gly Lys Arg Val Ser Tyr Glu Arg Thr Phe Ala Asn Glu
85 90 95

Cys Val Lys Asn Ser Tyr Gly Met Thr Val Phe Thr Phe
100 105

(2) INFORMATION FOR SEQ ID NO:35:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 113 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:35:

Asp Ala Pro Ser Ala Leu Tyr Ala Pro Ser Ala Leu Val Leu Thr Val
1 5 10 15

Gly Lys Gly Val Ser Ala Thr Thr Ala Ala Pro Glu Arg Ala Val Thr
20 25 30

Leu Thr Cys Ala Pro Gly Pro Ser Gly Thr His Pro Ala Ala Gly Ser
35 40 45

Ala Cys Ala Asp Leu Ala Ala Val Gly Gly Asp Leu Asn Ala Leu Thr
50 55 60

Arg Gly Glu Asp Val Met Cys Pro Met Val Tyr Asp Pro Val Leu Leu
65 70 75 80

Thr Val Asp Gly Val Trp Gln Gly Lys Arg Val Ser Tyr Glu Arg Val
85 90 95

Phe Ser Asn Glu Cys Glu Met Asn Ala His Gly Ser Ser Val Phe Ala
100 105 110

Phe

(2) INFORMATION FOR SEQ ID NO:36:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:36:

Tyr Ala Pro Ser Ala Leu Val Leu Thr Val Gly Lys Gly Val Ser Ala
1 5 10 15

Ala Thr Val Thr Pro Glu Arg Ala Val Thr Leu Thr Cys Ala Pro Gly
20 25 30

Pro Ser Gly Thr His Pro Ala Ala Asp Ser Ala Cys Ala Asp Leu Ala
35 40 45

Ala Val Gly Gly Asp Leu Asp Ala Leu Thr Arg Ser Glu Gly Val Met
50 55 60

Cys Pro Met Ile Tyr Asp Pro Val Leu Leu Thr Val Asp Gly Val Trp
65 70 75 80

Gln Gly Glu Arg Val Ser Tyr Glu Arg Val Phe Ser Asn Glu Cys Glu
85 90 95

Met Asn Ala His Gly Ser Ser Val Leu Ala Phe
100 105

(2) INFORMATION FOR SEQ ID NO:37:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:37:

```
His Ala Pro Asn Ala Leu Val Leu Thr Val Ala Lys Gly Glu Thr Ala
1               5                  10                  15

Arg Thr Ala Thr Pro Leu Arg Ala Val Thr Leu Thr Cys Ala Pro Thr
            20                  25                  30

Pro Gly Gly Thr His Pro Ala Pro Glu Ala Ala Cys Ala Glu Leu Arg
        35                  40                  45

Ala Val Asp Gly Arg Phe Ser Ala Leu Arg Gly Asp Gln Asp Arg Ala
    50                  55                  60

Cys Ile Lys Ile Tyr Asp Pro Leu Val Val Thr Ala Glu Gly Val Trp
65                  70                  75                  80

Glu Gly Gln Arg Val Arg Tyr Glu Arg Thr Phe Gly Asn Ser Cys Thr
                85                  90                  95

Leu Gln Thr Glu Ala Gly Pro Val Phe Ser Phe
            100                 105
```

(2) INFORMATION FOR SEQ ID NO:38:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 109 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:38:

```
Ser Leu His Ala Pro Ser Ala Leu Val Leu Thr Val Gly His Gly Glu
1               5                  10                  15

Ser Ala Ala Thr Ala Val Pro Leu Arg Ala Val Thr Leu Thr Cys Ala
            20                  25                  30

Pro Thr Ala Ser Gly Thr His Pro Ala Thr Val Ser Ala Cys Ala Glu
        35                  40                  45

Leu Arg Gly Ala Gly Gly Asp Phe Asp Ala Leu Ala Ala Asp Ala Gly
    50                  55                  60

Val Met Cys Thr Arg Glu Tyr Ala Pro Val Val Val Thr Val Asp Gly
65                  70                  75                  80

Val Trp Gln Gly Arg Arg Leu Ser Tyr Glu Arg Thr Phe Ala Asn Glu
                85                  90                  95
```

Gly Val Lys Asn Ala Gly Ser Ser Ser Val Phe Thr Phe
100 105

(2) INFORMATION FOR SEQ ID NO:39:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:39:

Tyr Ala Pro Ser Ala Leu Val Leu Thr Val Gly His Gly Glu Ser Ala
1 5 10 15

Ile Ala Ala Thr Pro Glu Arg Ala Val Thr Leu Thr Cys Ala Pro Lys
20 25 30

Ala Ala Gly Thr His Pro Ala Ala Gly Ala Ala Cys Ala Glu Leu Arg
35 40 45

Gly Val Gly Gly Asp Phe Asp Ala Leu Thr Ala Arg Asp Gly Val Met
50 55 60

Cys Thr Lys Gln Tyr Asp Pro Val Val Val Thr Val Glu Gly Val Trp
65 70 75 80

Gln Gly Lys Arg Val Ser Tyr Glu Arg Thr Phe Ser Asn Asp Cys Met
85 90 95

Lys Asn Ala Tyr Gly Thr Gly Val Phe Ser Phe
100 105

(2) INFORMATION FOR SEQ ID NO:40:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 111 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:40:

Ser Leu Tyr Pro Ala Ser Ala Leu Val Leu Thr Val Gly His Gly Ala
1 5 10 15

Asp Ala Ala Thr Ala Glu Val Gln Arg Ala Val Thr Leu Ser Cys Arg
20 25 30

Pro Thr Pro Thr Gly Thr His Pro Ala Pro Ala Gln Ala Cys Ala Glu
35 40 45

Leu His Ser Val Gly Gly Ala Leu Gly Leu Leu Arg Thr Gly Ala Glu
50 55 60

Pro Gly Arg Met Cys Thr Lys Glu Trp Arg Pro Ile Thr Val Thr Ala
65 70 75 80

Glu Gly Val Trp Asp Gly Arg Arg Val Ser Tyr Glu His Thr Phe Ala
85 90 95

Asn Asn Cys Phe Lys Asn Ala Ala Pro Thr Thr Val Phe Glu Phe
100 105 110

(2) INFORMATION FOR SEQ ID NO:41:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 108 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:41:

Tyr Ala Pro Ser Ala Leu Val Leu Thr Val Gly Glu Gly Glu Ser Ala
1 5 10 15

Ala Ala Ala Thr Pro Glu Arg Ala Val Thr Leu Thr Cys Ala Pro Arg
20 25 30

Pro Ser Gly Thr His Pro Val Ala Gly Ser Ala Cys Ala Glu Leu Arg
35 40 45

Gly Val Gly Gly Asp Val Gly His Ala Leu Thr Ala Thr Asp Gly Val
50 55 60

Met Cys Thr Lys Gln Tyr Asp Pro Val Val Val Thr Val Asp Gly Val
65 70 75 80

Trp Gln Gly Arg Arg Val Ser Tyr Glu Arg Thr Phe Ser Asn Glu Cys
85 90 95

Val Lys Asn Ala Tyr Gly Ser Gly Val Phe Ala Phe
100 105

(2) INFORMATION FOR SEQ ID NO:42:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 107 amino acids

(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:42:

Tyr Ala Pro Ser Ala Leu Val Leu Thr Val Gly His Gly Glu Ser Ala
1 5 10 15

Ala Thr Ala Ala Pro Leu Arg Ala Val Thr Leu Thr Cys Ala Pro Thr
20 25 30

Ala Ser Gly Thr His Pro Ala Ala Ala Ala Ala Cys Ala Glu Leu Arg
35 40 45

Ala Ala His Gly Asp Pro Ser Ala Leu Ala Ala Glu Asp Ser Val Met
50 55 60

Cys Thr Arg Glu Tyr Ala Pro Val Val Val Thr Val Asp Gly Val Trp
65 70 75 80

Gln Gly Arg Arg Leu Ser Tyr Glu Arg Thr Phe Ala Asn Glu Cys Val
85 90 95

Lys Asn Ala Gly Ser Ala Ser Val Phe Thr Phe
100 105

(2) INFORMATION FOR SEQ ID NO:43:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:43:

Cys Thr Arg Xaa Tyr Xaa Pro Val Val Val Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:44:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 10 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:44:

Cys Thr Lys Tyr Xaa Pro Val Val Val Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:45:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:45:

Cys Asn Arg Xaa Tyr Xaa Pro Val Val Val Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:46:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:46:

Cys Asn Lys Xaa Tyr Xaa Pro Val Val Val Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:47:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:47:

Cys Thr Arg Xaa Trp Xaa Pro Val Val Val Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:48:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 11 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:48:

Cys Thr Lys Xaa Trp Xaa Pro Val Val Val Thr
1 5 10

(2) INFORMATION FOR SEQ ID NO:49:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:49:

Ala Leu Glu Gly Ser Leu Gln Lys Arg Gly Ile Val Glu Gln Cys Cys
1 5 10 15

(2) INFORMATION FOR SEQ ID NO:50:

(i) SEQUENCE CHARACTERISTICS:
(A) LENGTH: 16 amino acids
(B) TYPE: amino acid
(C) STRANDEDNESS: single
(D) TOPOLOGY: linear

(ii) MOLECULE TYPE: peptide

(xi) SEQUENCE DESCRIPTION: SEQ ID NO:50:

Ala Leu Glu Gly Ser Leu Gln Lys His Gly Ile Val Glu Gln Cys Cys
1 5 10 15

WHAT IS CLAIMED IS:

1. A peptide inhibitor of propeptide/prohormone convertase selected from the group consisting of

CTRXYXPVVVT (SEQ ID NO: 43),
CTKXYXPVVVT (SEQ ID NO: 44),
CNRXYXPVVVT (SEQ ID NO: 45),
CNKXYXPVVVT (SEQ ID NO: 46),
CTRXWXPVVVT (SEQ ID NO: 47),
CTKXWXPVVVT (SEQ ID NO: 48),

wherein X is any of the 20 common amino acids,

and longer peptides containing said peptides.

2. A peptide inhibitor of propeptide/prohormone convertase selected from the group consisting of:

RGDVACTKQFDPVVVT (SEQ ID NO: 1),
GEDVMCPMVYDPVLLT (SEQ ID NO: 2),
DTGRVCTREYRPVTVS (SEQ ID NO: 3),
RDGVICNKLYDPVVVT (SEQ ID NO: 4),
ADGVMCTREYAPVVVT (SEQ ID NO: 5),
SEGVMCPMIYDPVLLT (SEQ ID NO: 6),
CALEGSLQKRGIVEQCC (SEQ ID NO: 7),
CALEGSLQKHGIVEQCC (SEQ ID NO: 8),
CRDGVICNKLYDPVVVTC (SEQ ID NO: 9),
CADGVMCTREYAPVVVTC (SEQ ID NO: 10),
CDTGRVCTREYRPVTVSC (SEQ ID NO: 11),
CRGDVACTKQFDPVVVTC (SEQ ID NO: 13),
CGEDVMCPMVYDPVLLTC (SEQ ID NO: 14),
CSEGVMCPMIYDPVLLTC (SEQ ID NO: 15),
RDGVICNKNYDPVVVT (SEQ ID NO: 16),
DDAVMCTREYAPVVVT (SEQ ID NO: 17),
DQDRACIKIYDPLVVT (SEQ ID NO: 18),

DAGVMCTREYAPVVVT (SEQ ID NO: 19),
RDGVMCTKQYDPVVVT (SEQ ID NO: 20),
EPGRMCTKEWRPITVT (SEQ ID NO: 21),
RDDVWCNKLYDPVVVT (SEQ ID NO: 22),
EDSVMCTREYAPVVVT (SEQ ID NO: 23),
TGDVMCTKQYDVVVT (SEQ ID NO: 24),

and longer peptides containing said peptides.

3. A peptide inhibitor of the following sequence:

ASLYAPSALVLTVGHGTSAAAA
SPLRAVTLNCAPTASGTHPAPALA
CADLRGVGGDIDALKARDGVICNKL
YDPVVVTVDGVWQGKRVSYERTFGNE
CVKNSYGTSLFAF, (SEQ ID NO:25)

and derivatives and mutants thereof.

4. The peptide inhibitor according to Claims 1 or 2, where said propeptide/prohormone convertase is selected from the group consisting of PC1/3, PC2, PC6A, PC7 and furin.

5. The peptide inhibitor according to Claims 1 or 2, wherein said peptide inhibitor is useful in the treatment of a tumor cell.

6. The peptide inhibitor according to Claim 5, wherein said tumor cell is a hormone-secreting tumor cell.

7. The peptide inhibitor according to Claim 5, wherein said tumor cell is a tumor cell of a neuroendocrine neoplastic disease.

8. The peptide inhibitor of Claim 5, wherein said tumor cell is a tumor cell of a neoplastic disease involving overproduction of a growth factor.

9. The peptide inhibitor according to Claims 1 or 2, wherein said peptide inhibitor is useful in the treatment of a cell infected with a viral pathogen.

10. The peptide inhibitor according to Claim 9, wherein said peptide inhibitor is useful in the treatment of a cell infected with HIV.

11. A method of suppressing propeptide/prohormone convertase activity comprising the steps of treating a propeptide/prohormone convertase with a peptide selected from the group consisting of

CTRXYXPVVVT (SEQ ID NO: 43),
CTKXYXPVVVT (SEQ ID NO: 44),
CNRXYXPVVVT (SEQ ID NO: 45),
CNKXYXPVVVT (SEQ ID NO: 46),
CTRXWXPVVVT (SEQ ID NO: 47),
CTKXWXPVVVT (SEQ ID NO: 48),

wherein X is any of the 20 common amino acids,

and longer peptides containing said peptides.

12. A method of suppressing propeptide/prohormone convertase acitivity comprising the steps of treating a propeptide/prohormone convertase with a peptide selected from the group consisting of

RGDVACTKQFDPVVVT (SEQ ID NO: 1),
GEDVMCPMVYDPVLLT (SEQ ID NO: 2),
DTGRVCTREYRPVTVS (SEQ ID NO: 3),
RDGVICNKLYDPVVVT (SEQ ID NO: 4),
ADGVMCTREYAPVVVT (SEQ ID NO: 5),
SEGVMCPMIYDPVLLT (SEQ ID NO: 6),
CALEGSLQKRGIVEQCC (SEQ ID NO: 7),
CALEGSLQKHGIVEQCC (SEQ ID NO: 8),
CRDGVICNKLYDPVVVTC (SEQ ID NO: 9),
CADGVMCTREYAPVVVTC (SEQ ID NO: 10),
CDTGRVCTREYRPVTVSC (SEQ ID NO: 11),
CRGDVACTKQFDPVVVTC (SEQ ID NO: 13),
CGEDVMCPMVYDPVLLTC (SEQ ID NO: 14),
CSEGVMCPMIYDPVLLTC (SEQ ID NO: 15),
RDGVICNKNYDPVVVT (SEQ ID NO: 16),

DDAVMCTREYAPVVVT (SEQ ID NO: 17),
DQDRACIKIYDPLVVT (SEQ ID NO: 18),
DAGVMCTREYAPVVVT (SEQ ID NO: 19),
RDGVMCTKQYDPVVVT (SEQ ID NO: 20),
EPGRMCTKEWRPITVT (SEQ ID NO: 21),
RDDVWCNKLYDPVVVT (SEQ ID NO: 22),
EDSVMCTREYAPVVVT (SEQ ID NO: 23),
TGDVMCTKQYDVVVT (SEQ ID NO: 24),

and longer peptides containing said peptides, and monitoring the degree of inhibition of propeptide/prohormone convertases.

13. The method according to claims 11 or 12, where said propeptide/prohormone convertase is selected from the group consisting of PC1/3, PC2, PC6A, PC7, and furin.

14. The method of treating a tumor cell, comprising the steps of treating a tumor cell with at least one peptide selected from the group consisting of

CTRXYXPVVVT (SEQ ID NO: 43),
CTKXYXPVVVT (SEQ ID NO: 44),
CNRXYXPVVVT (SEQ ID NO: 45),
CNKXYXPVVVT (SEQ ID NO: 46),
CTRXWXPVVVT (SEQ ID NO: 47),
CTKXWXPVVVT (SEQ ID NO: 48),

wherein X is any of the 20 common amino acids,

and longer peptides containing said peptides.

15. A method of treating a tumor cell comprising the steps of treating a tumor cell with at least one peptide selected from the group consisting of

RGDVACTKQFDPVVVT (SEQ ID NO: 1),
GEDVMCPMVYDPVLLT (SEQ ID NO: 2),
DTGRVCTREYRPVTVS (SEQ ID NO: 3),
RDGVICNKLYDPVVVT (SEQ ID NO: 4),

ADGVMCTREYAPVVVT (SEQ ID NO: 5),
SEGVMCPMIYDPVLLT (SEQ ID NO: 6),
CALEGSLQKRGIVEQCC (SEQ ID NO: 7),
CALEGSLQKHGIVEQCC (SEQ ID NO: 8),
CRDGVICNKLYDPVVVTC (SEQ ID NO: 9),
CADGVMCTREYAPVVVTC (SEQ ID NO: 10),
CDTGRVCTREYRPVTVSC (SEQ ID NO: 11),
CRGDVACTKQFDPVVVTC (SEQ ID NO: 13),
CGEDVMCPMVYDPVLLTC (SEQ ID NO: 14),
CSEGVMCPMIYDPVLLTC (SEQ ID NO: 15),
RDGVICNKNYDPVVVT (SEQ ID NO: 16),
DDAVMCTREYAPVVVT (SEQ ID NO: 17),
DQDRACIKIYDPLVVT (SEQ ID NO: 18),
DAGVMCTREYAPVVVT (SEQ ID NO: 19),
RDGVMCTKQYDPVVVT (SEQ ID NO: 20),
EPGRMCTKEWRPITVT (SEQ ID NO: 21),
RDDVWCNKLYDPVVVT (SEQ ID NO: 22),
EDSVMCTREYAPVVVT (SEQ ID NO: 23),
TGDVMCTKQYDVVVT (SEQ ID NO: 24),

and longer peptides containing said peptides; and monitoring the degree of inhibition of propeptide/prohormone convertase.

16. The method according to claims 14 or 15, where said propeptide/prohormone convertase is PC1/3.

17. The method according to claims 14 or 15, where said propeptide/prohormone convertase is PC2.

18. The method according to claims 14 or 15, wherein said tumor cell is a hormone-secreting tumor cell.

19. The method according to claims 14 or 15, wherein said tumor cell is a tumor cell of a neuroendocrine neoplastic disease.

20. The method according to claim 14 or 15, wherein said tumor cell is a tumor cell of a neoplastic disease involving overproduction of a growth factor.

21. A method for treating a cell infected with a viral pathogen with at least one peptide from the group consisting of

CTRXYXPVVVT (SEQ ID NO: 43),
CTKXYXPVVVT (SEQ ID NO: 44),
CNRXYXPVVVT (SEQ ID NO: 45),
CNKXYXPVVVT (SEQ ID NO: 46),
CTRXWXPVVVT (SEQ ID NO: 47),
CTKXWXPVVVT (SEQ ID NO: 48),

wherein X is any of the 20 common amino acids,

and longer peptides containing said peptides.

22. A method of treating a cell infected with a viral pathogen with at least one peptide from the group consisting of

RGDVACTKQFDPVVVT (SEQ ID NO: 1),
GEDVMCPMVYDPVLLT (SEQ ID NO: 2),
DTGRVCTREYRPVTVS (SEQ ID NO: 3),
RDGVICNKLYDPVVVT (SEQ ID NO: 4),
ADGVMCTREYAPVVVT (SEQ ID NO: 5),
SEGVMCPMIYDPVLLT (SEQ ID NO: 6),
CALEGSLQKRGIVEQCC (SEQ ID NO: 7),
CALEGSLQKHGIVEQCC (SEQ ID NO: 8),
CRDGVICNKLYDPVVVTC (SEQ ID NO: 9),
CADGVMCTREYAPVVVTC (SEQ ID NO: 10),
CDTGRVCTREYRPVTVSC (SEQ ID NO: 11),
CRGDVACTKQFDPVVVTC (SEQ ID NO: 13),
CGEDVMCPMVYDPVLLTC (SEQ ID NO: 14),
CSEGVMCPMIYDPVLLTC (SEQ ID NO: 15),
RDGVICNKNYDPVVVT (SEQ ID NO: 16),

DDAVMCTREYAPVVVT (SEQ ID NO: 17),
DQDRACIKIYDPLVVT (SEQ ID NO: 18),
DAGVMCTREYAPVVVT (SEQ ID NO: 19),
RDGVMCTKQYDPVVVT (SEQ ID NO: 20),
EPGRMCTKEWRPITVT (SEQ ID NO: 21),
RDDVWCNKLYDPVVVT (SEQ ID NO: 22),
EDSVMCTREYAPVVVT (SEQ ID NO: 23),
TGDVMCTKQYDVVVT (SEQ ID NO: 24),

and longer peptides containing said peptides; and monitoring the degree of inhibition of propeptide/prohormone convertase.

23. The method according to Claims 21 or 22, where said propeptide/prohormone is PC6A.

24. The method according to Claims 21 or 22, where said propeptide/prohormone is PC7.

25. The method according to Claims 21 or 22, where said propeptide/prohormone is furin.

26. The method according to Claims 21 or 22, wherein the cell is selected from the group consisting of lymphoid and monocyte/macrophage cells.

27. A nucleic acid encoding the peptide inhibitor of Claims 1 or 2.

28. An expression vector for a peptide inhibitor comprising at least one nucleic acid encoding at least one peptide inhibitor according to Claims 1 or 2, operatively linked to a nucleic acid encoding a signal peptide.

29. The expression vector of Claim 28, further comprising a nucleic acid encoding a Golgi retention signal.

30. The expression vector of Claim 28, further comprising a nucleic acid encoding an inducible element.

31. The expression vector of Claim 29, wherein the inducible element is a 5' LTR component of HIV-1.

32. A chimeric protein comprising at least one peptide inhibitor of Claims 1 or 2, fused at its amino terminus to a signal peptide.

33. An expression vector for a peptide inhibitor comprising at least one nucleic acid encoding at least one peptide of Claims 1 or 2, operatively linked to a nucleic acid encoding a carrier peptide.

34. The expression vector of Claim 33, wherein the carrier peptide is a preproregion of a prohormone.

35. The expression vector of Claim 34, wherein the preprohormone is prosomatostatin.

36. The expression vector of Claim 33, further comprising a nucleic acid encoding a Golgi retention signal.

37. The expression vector of Claim 33, further comprising a nucleic acid encoding an inducible element.

38. The expression vector of Claim 37, wherein the inducible element is a 5' LTR of HIV.

39. A chimeric protein comprising at least one peptide inhibitor of Claims 1 or 2, fused at its amino terminus to the preproregion of a prohormone.

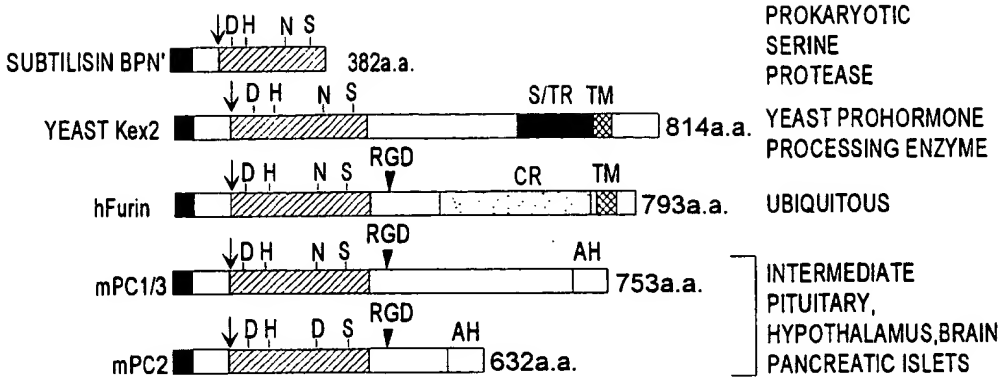

FIG. 1

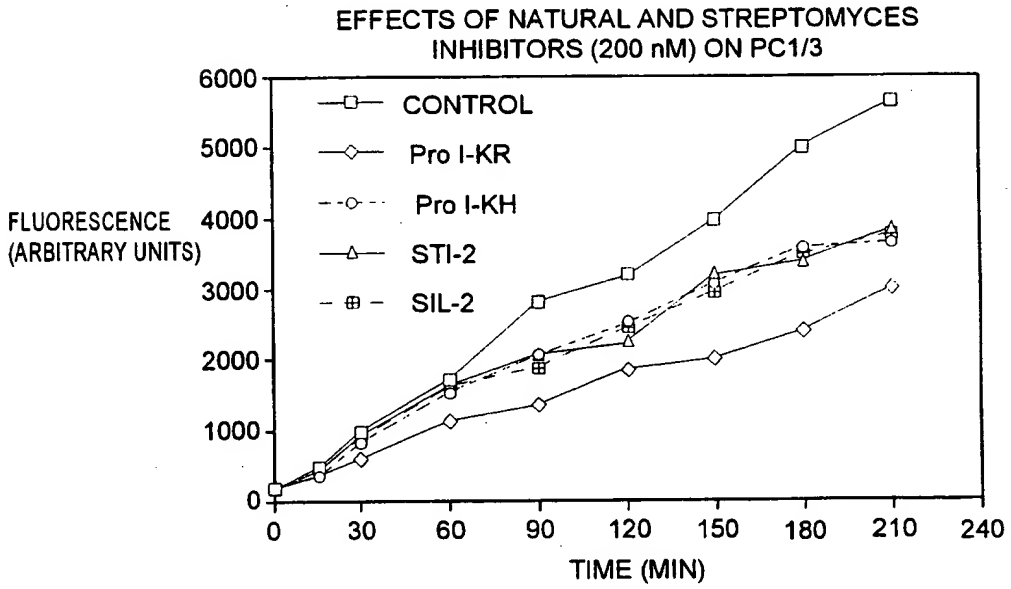

FIG. 3

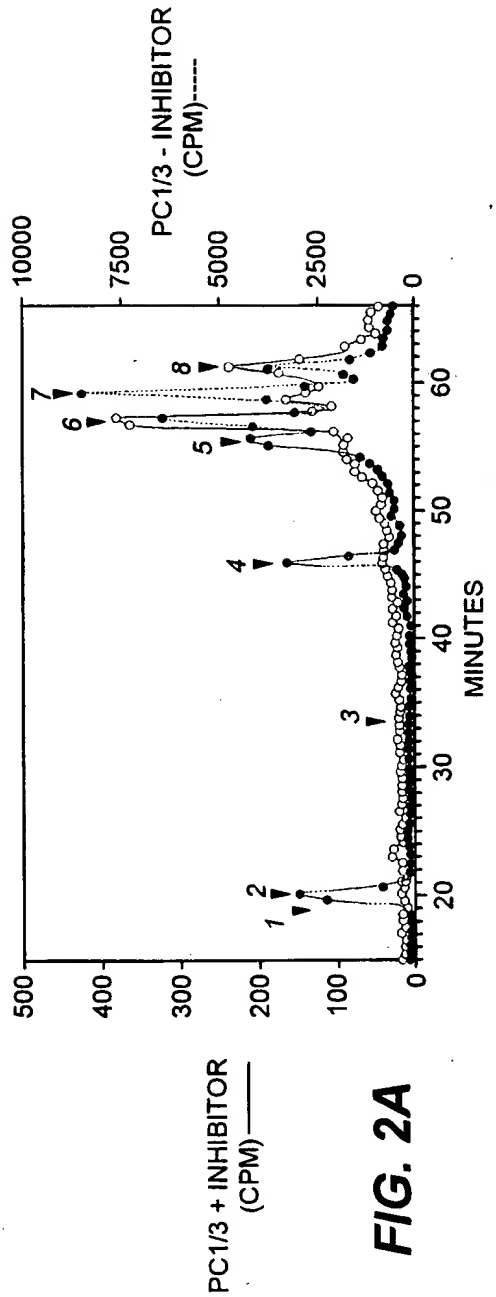

FIG. 2A

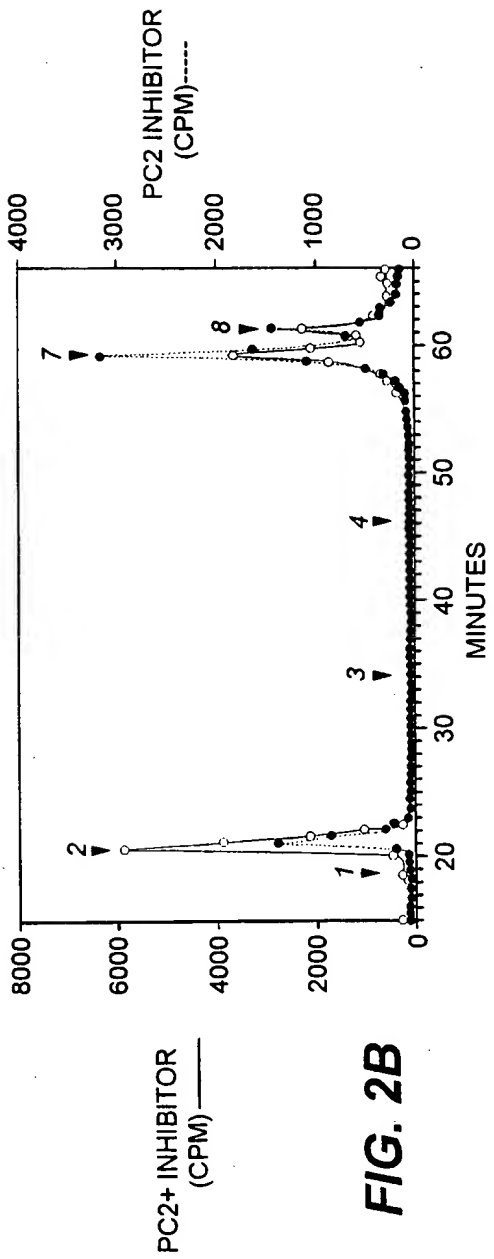

FIG. 2B

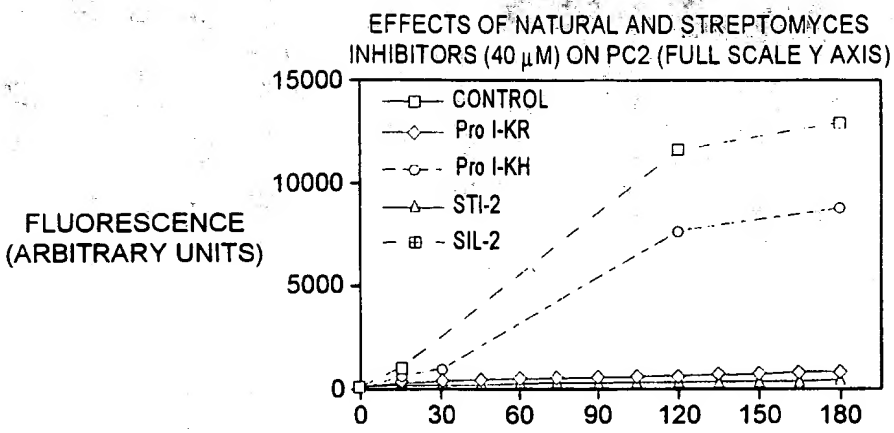

FIG. 4A

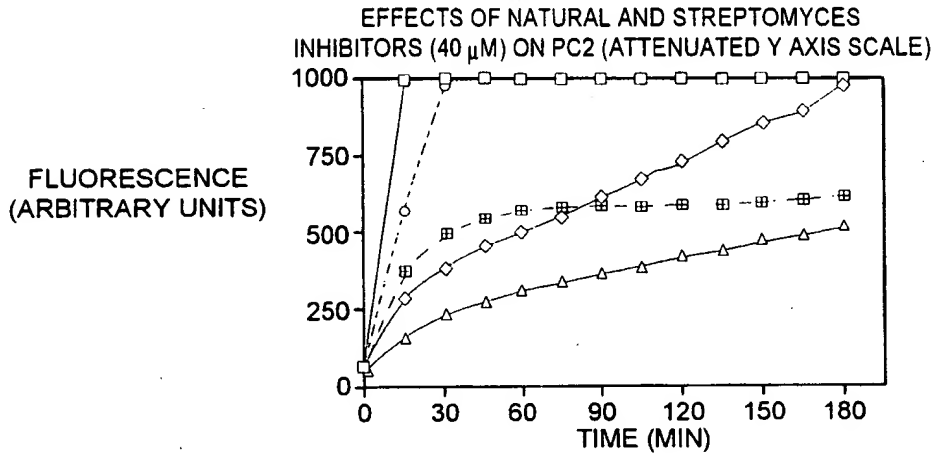

FIG. 4B

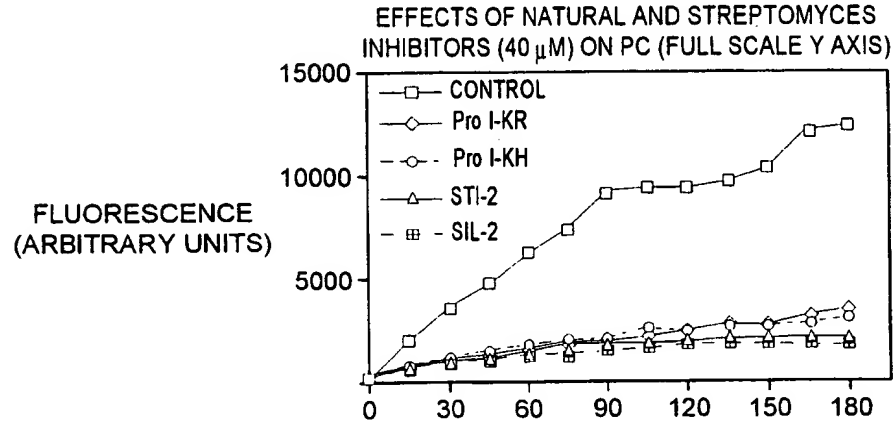

FIG. 4C

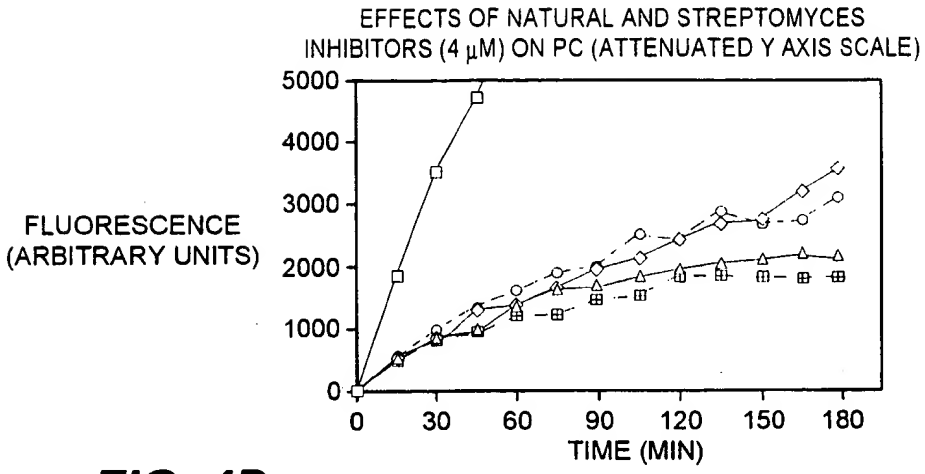

FIG. 4D

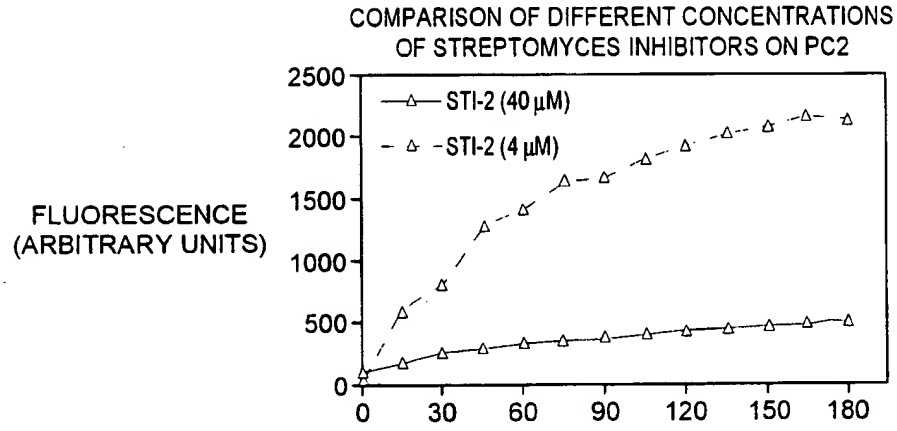

FIG. 4E

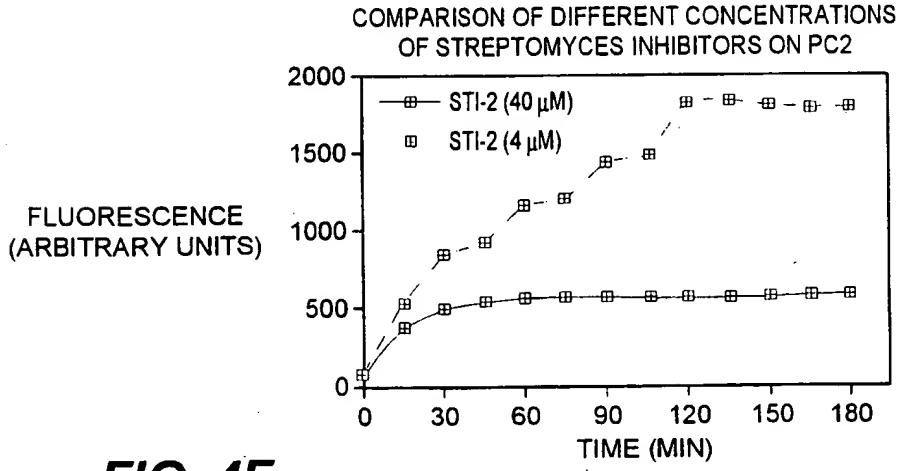

FIG. 4F

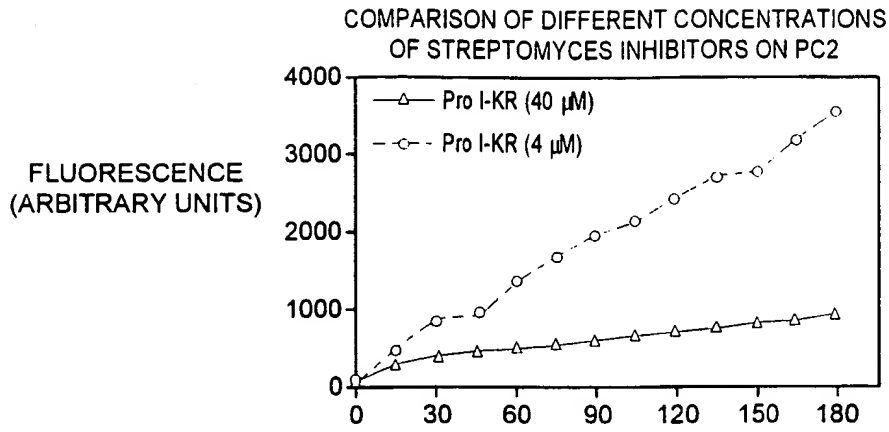

FIG. 4G

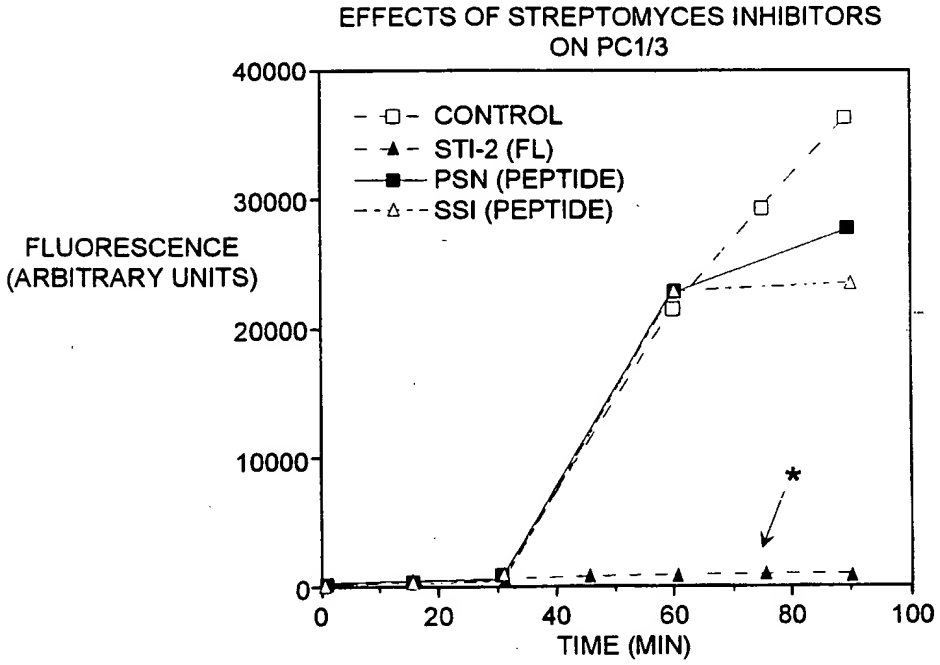

FIG. 5

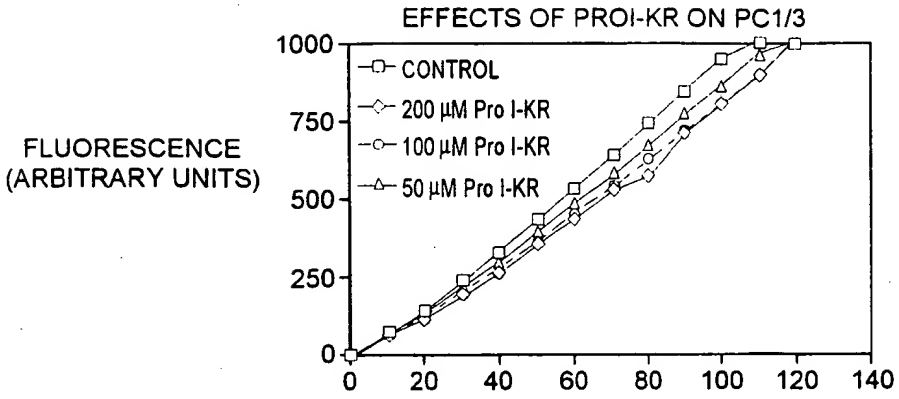

FIG. 6A

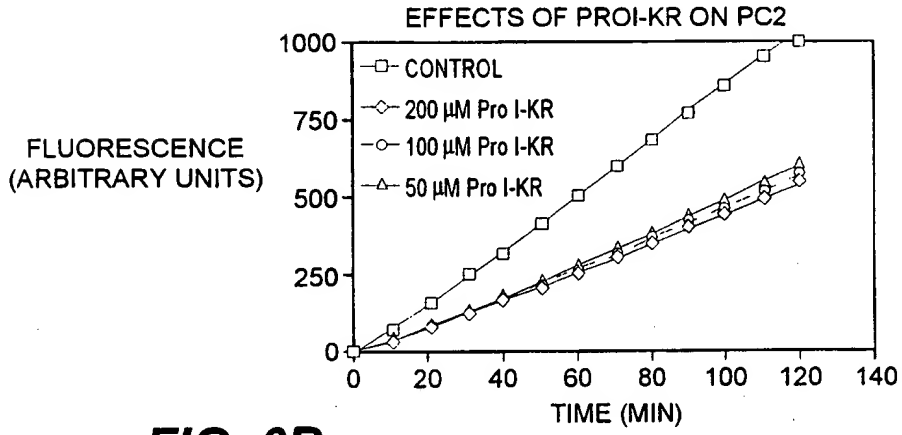

FIG. 6B

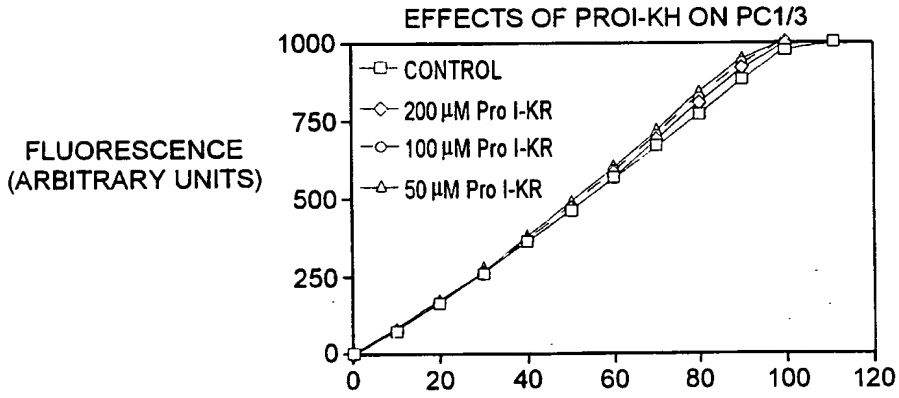

FIG. 6C

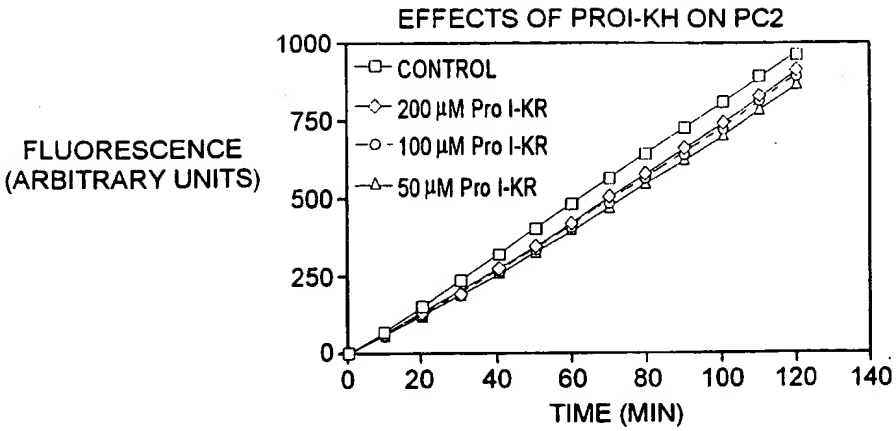

FIG. 6D

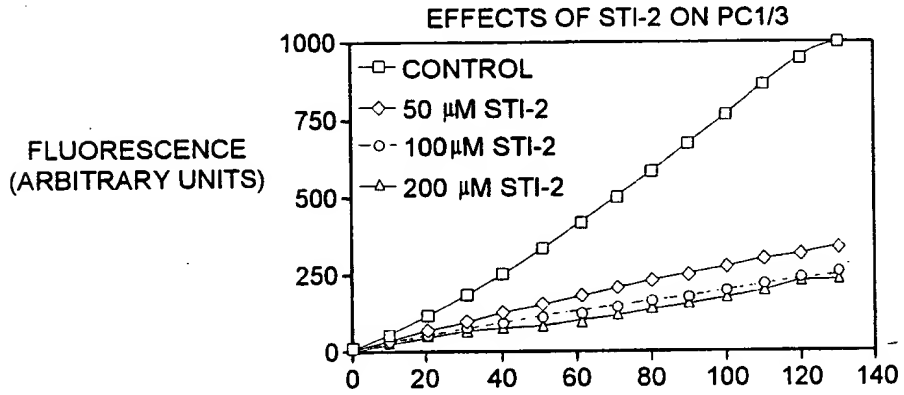

FIG. 7A

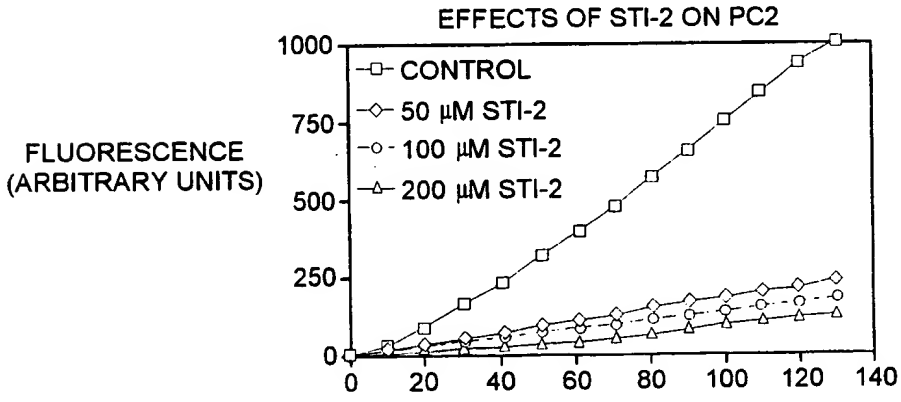

FIG. 7B

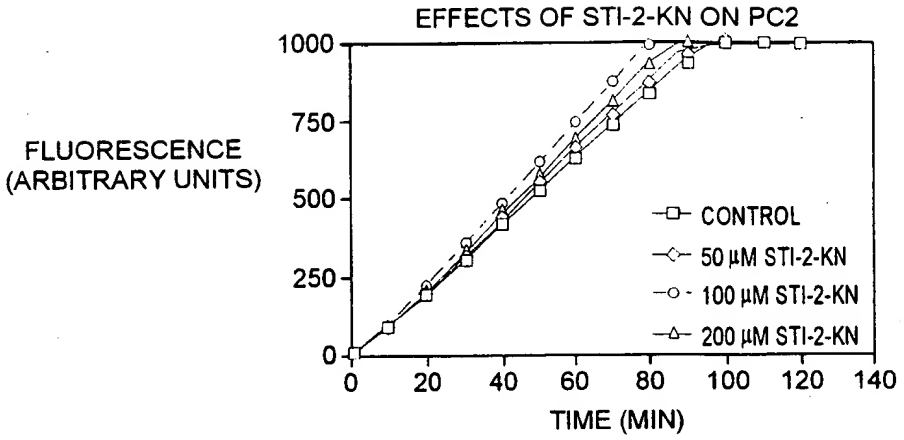

FIG. 7C

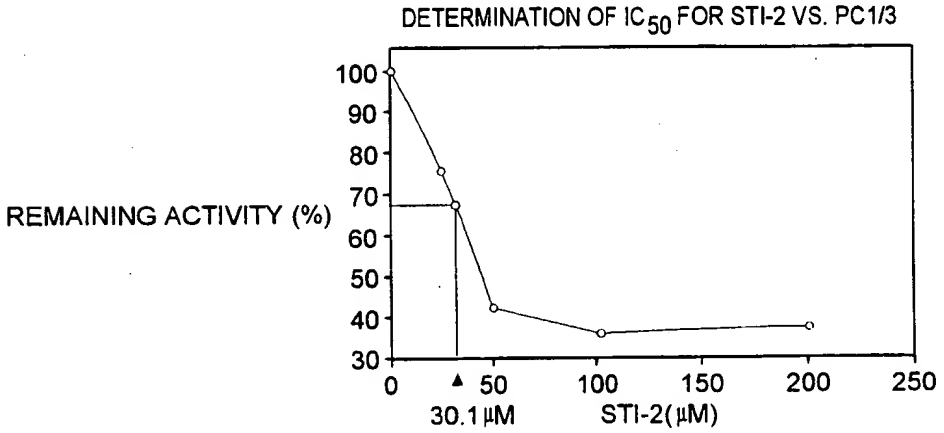

FIG. 7D

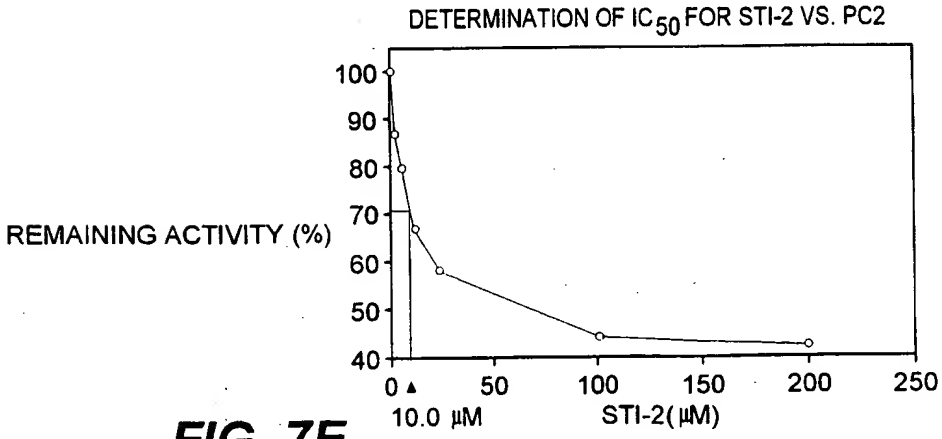

FIG. 7E

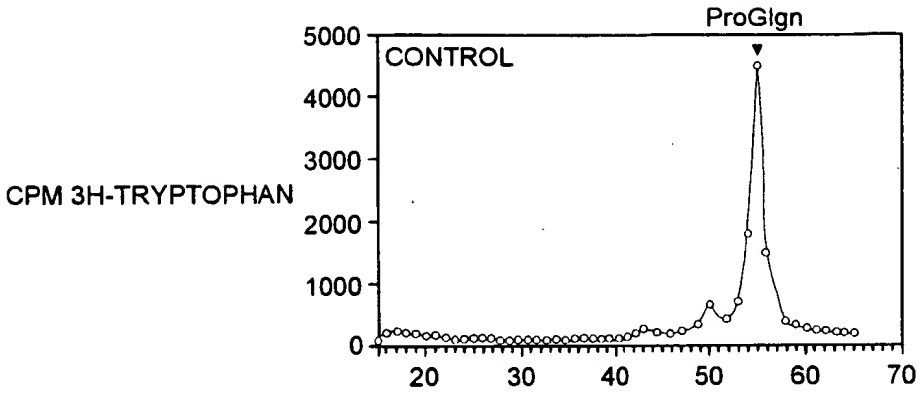

FIG. 8A

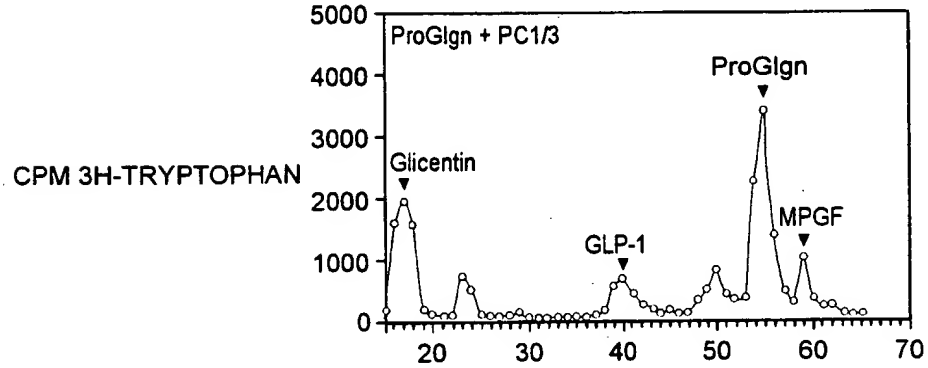

FIG. 8B

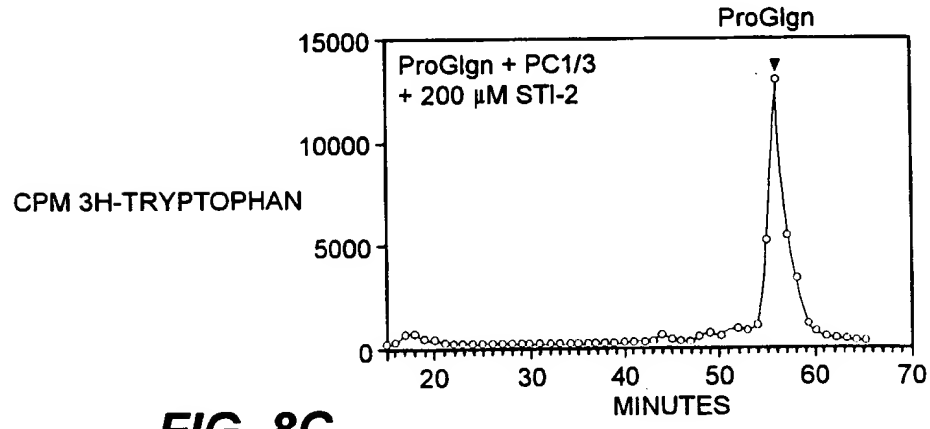

FIG. 8C

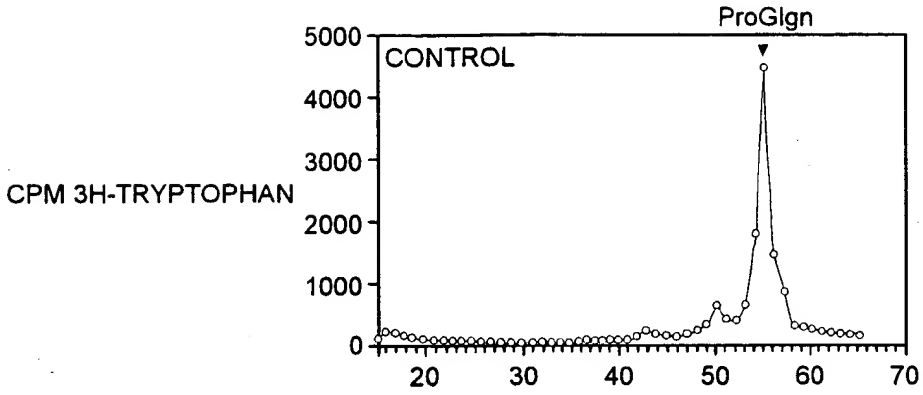

FIG. 8D

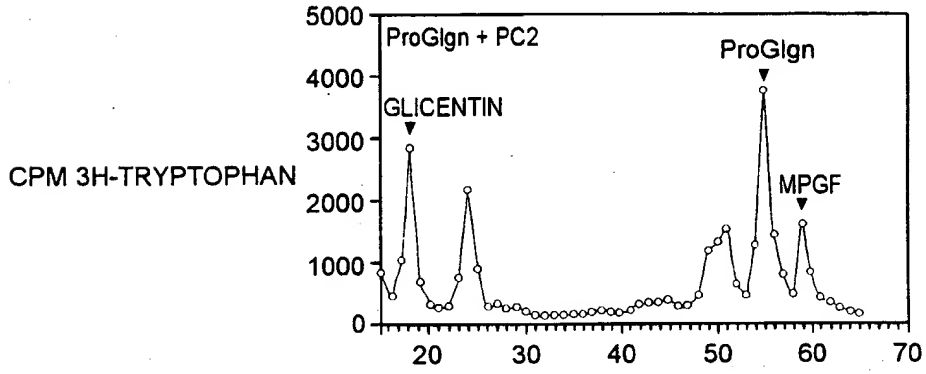

FIG. 8E

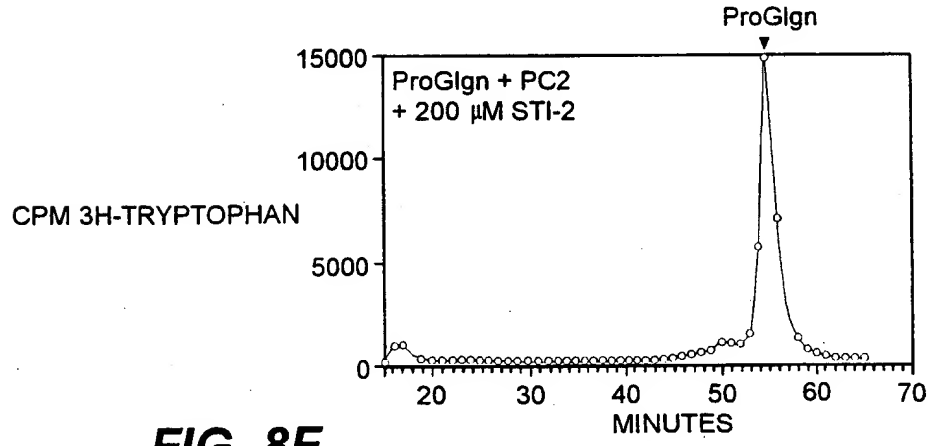

FIG. 8F

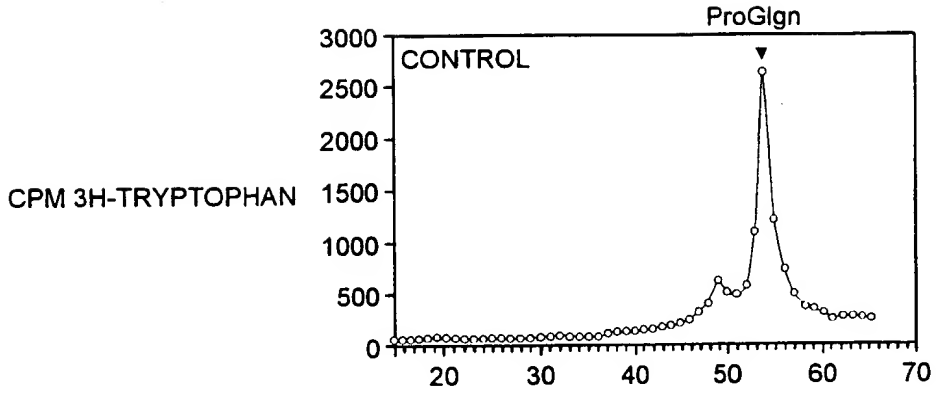

FIG. 9A

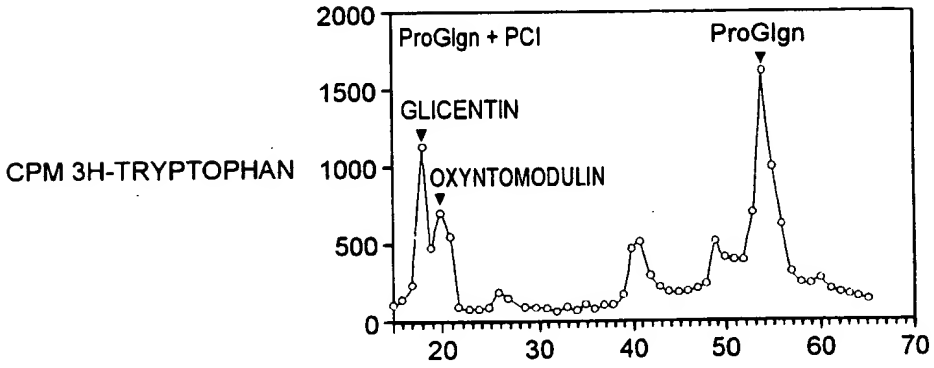

FIG. 9B

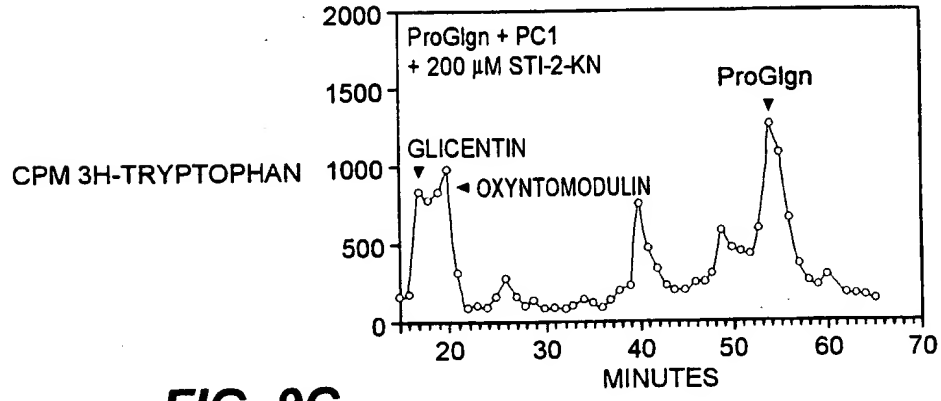

FIG. 9C

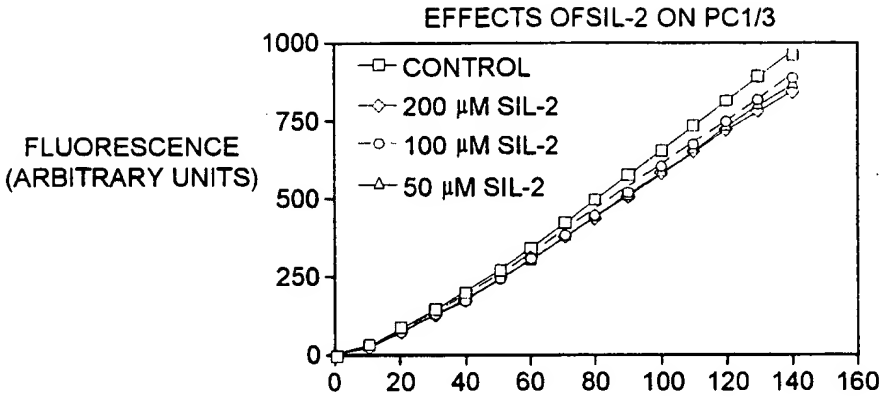

FIG. 9D

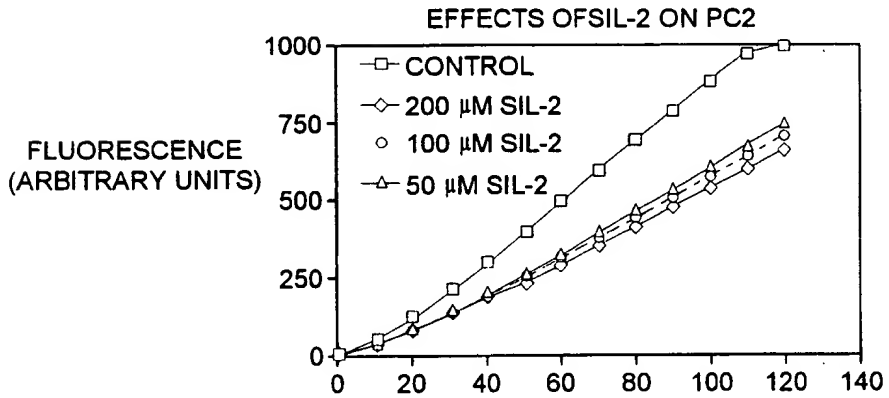

FIG. 9E

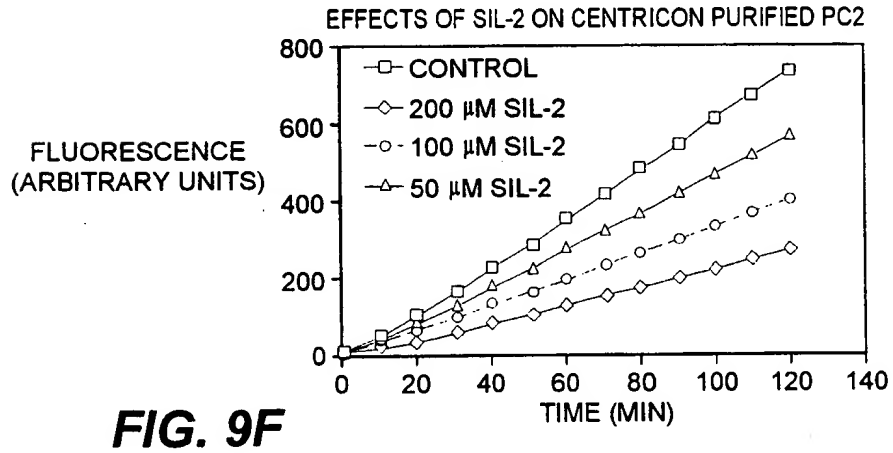

FIG. 9F

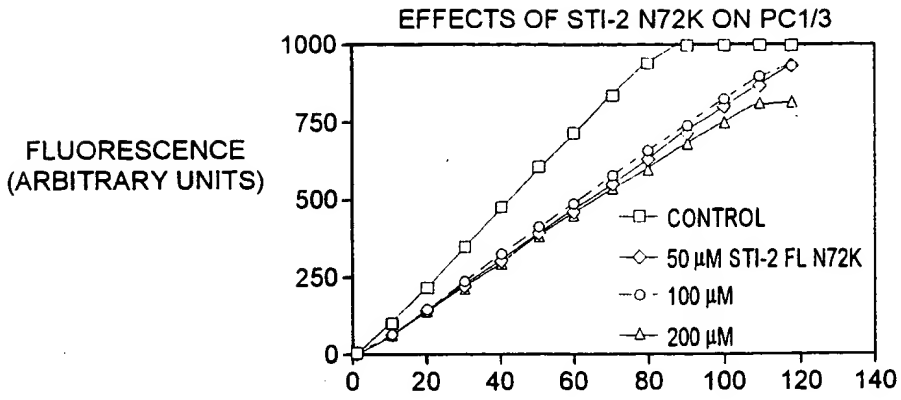

FIG. 10A

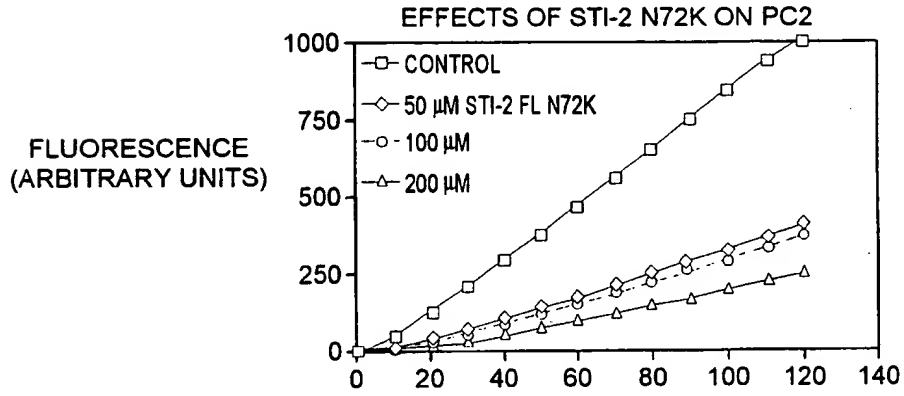

FIG. 10B

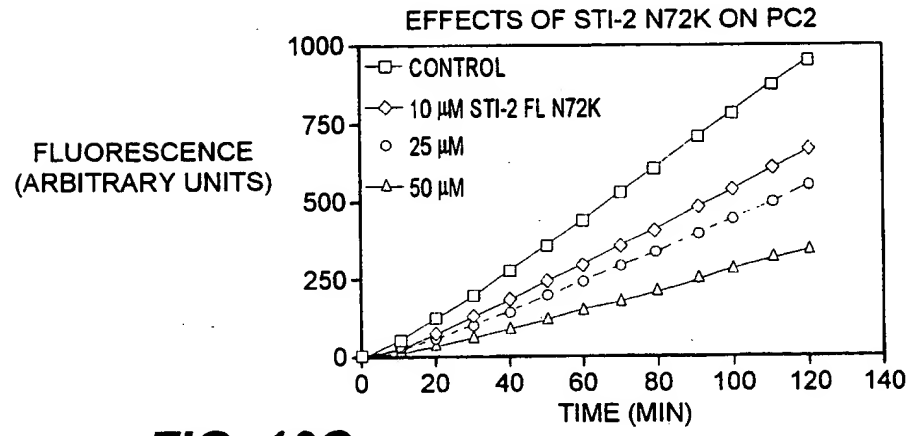

FIG. 10C

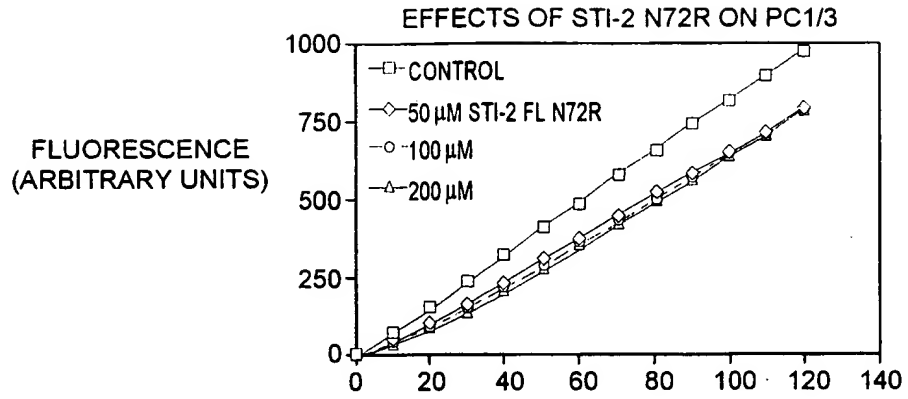

FIG. 10D

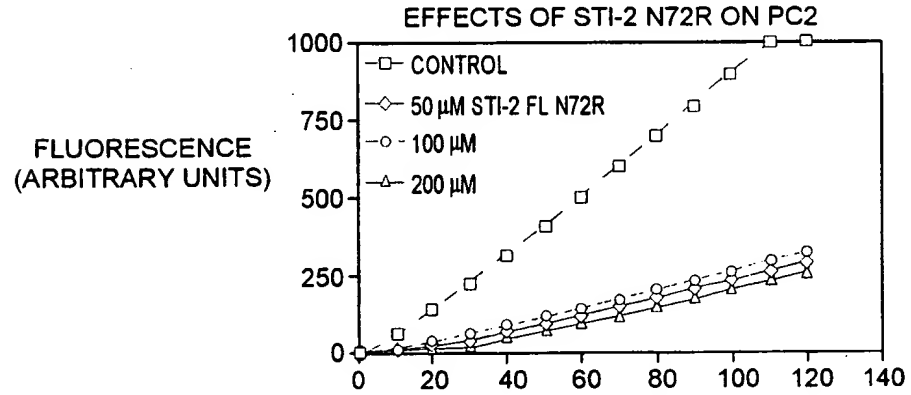

FIG. 10E

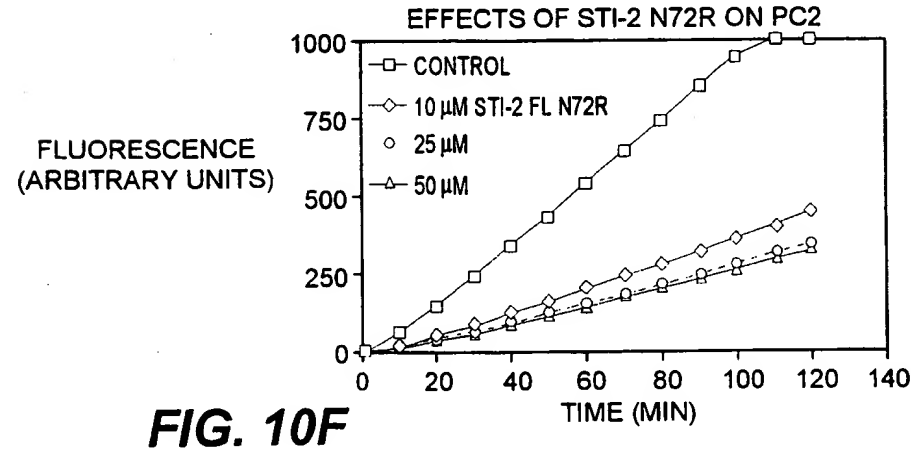

FIG. 10F

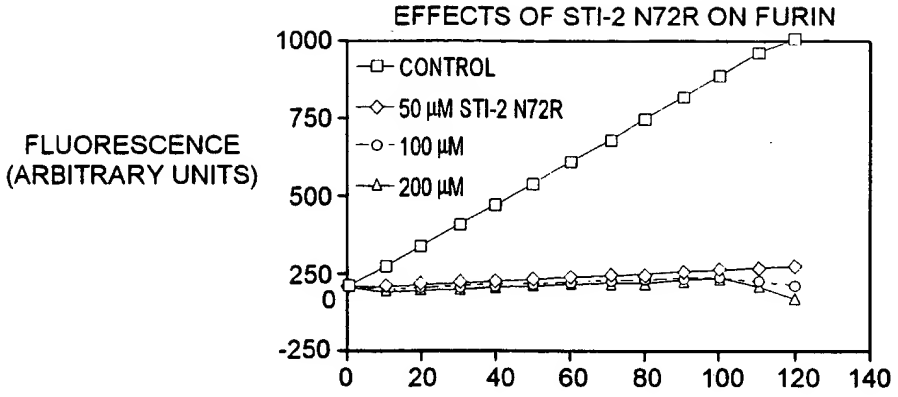

FIG. 11A

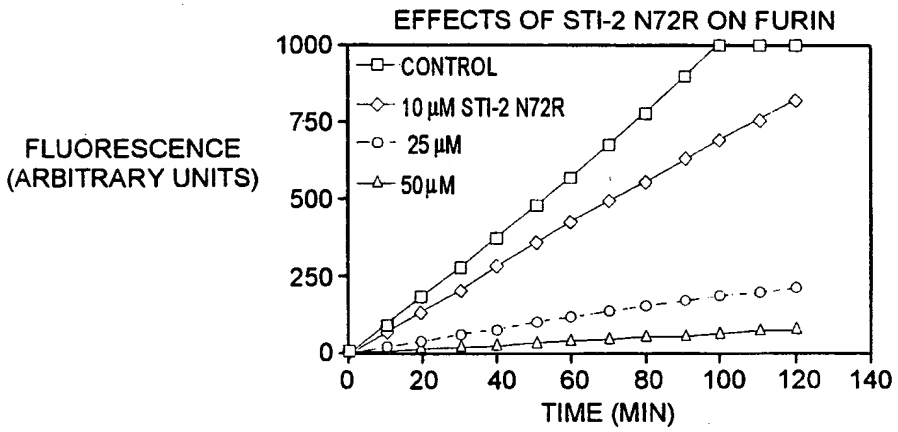

FIG. 11B

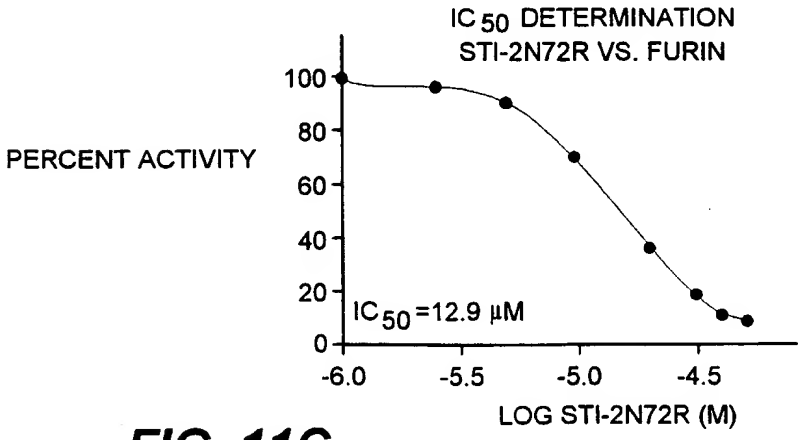

FIG. 11C

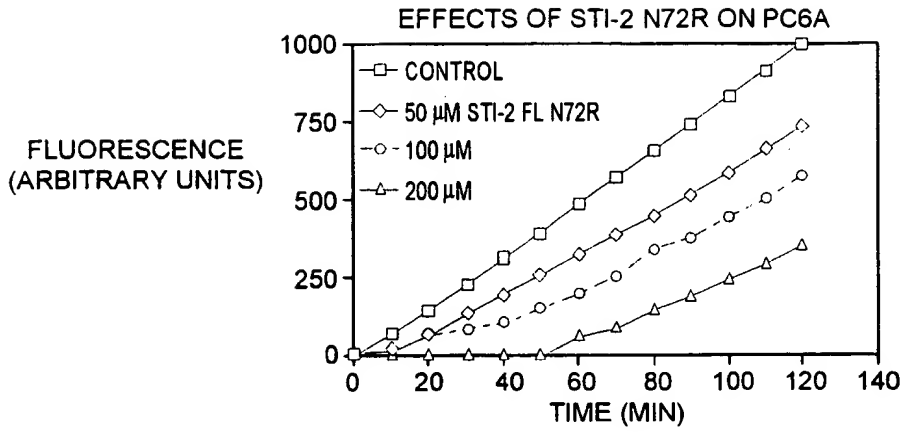

FIG. 11D

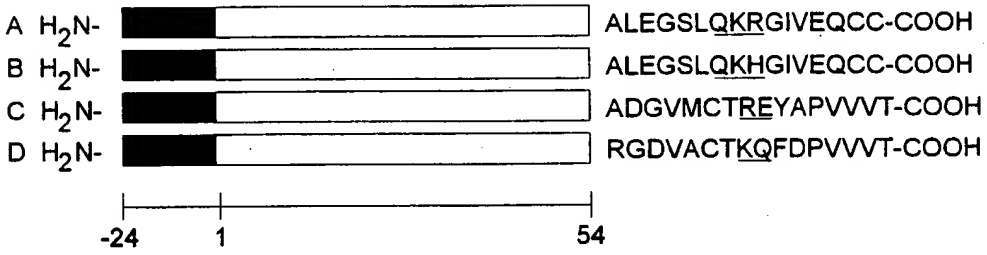

FIG. 12

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/03642

A. CLASSIFICATION OF SUBJECT MATTER

IPC(6) :A61K 38/55, 38/10, 38/03; C12N 9/50
US CL :930/250; 435/212, 219; 514/13, 14; 530/326
According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 930/250; 435/212, 219; 514/13, 14; 530/326

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)

Please See Extra Sheet.

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	WO 93/17086 A1(NOVO NORDISK A/S (DK/DK) 02 September 1993, pages 14, 17, and 18, has sequences matching SEQ ID NO:1.	2, 4-10 --- 1, 11-20
X --- Y	US 5,498,529 A (BERKA et al.) 12 March 1996, cols 3/4, 7/8 and 9/10 has sequences matching SEQ ID NOs: 4, 23, 43 and 46.	1, 2, 4-10 --- 3, 11-20
X --- Y	TAGUCHI, S. et al. Comparative Studies on the Primary Structures and Inhibitory Properties of Subtilisin-trypsin Inhibitors from Streptomyces. Eur. J. Biochem, 1994, volume 220, pages 911-918, see Figs 2 and 3. Has sequences matching SEQ ID NO: 5, 17, 22, 43 and 46.	1, 2, 4-10, --- 11-20

[X] Further documents are listed in the continuation of Box C. [ ] See patent family annex.

*	Special categories of cited documents:	"T"	later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention
"A"	document defining the general state of the art which is not considered to be of particular relevance	"X"	document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone
"E"	earlier document published on or after the international filing date	"Y"	document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art
"L"	document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)	"&"	document member of the same patent family
"O"	document referring to an oral disclosure, use, exhibition or other means		
"P"	document published prior to the international filing date but later than the priority date claimed		

Date of the actual completion of the international search 25 JUNE 1998	Date of mailing of the international search report 0 8 JUL 1998
Name and mailing address of the ISA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer MARY K. ZEMAN Telephone No. (703) 308-0196

Form PCT/ISA/210 (second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/03642

C (Continuation). DOCUMENTS CONSIDERED TO BE RELEVANT

Category*	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X --- Y	KOJIMA, S. et al. Primary Structure and Inhibitory Properties of a Proteinase inhibitor produced by Streptomyces cacaoi. Biochim. et. Biophys. Acta. 1994. Vol. 1207, pages 120-125. See figures 3 and 4. Has sequences matching SEQ ID NO: 3.	2, 4-10 --- 12, 13, 15-20
X --- Y	TERABE M. et al. New Subtilisin-trypsin Inhibitors produced by Streptomyces: Primary Structures and their Relationship to other Proteinase Inhibitors from Streptomyces. Biochim. et. Biophys. Acta. 1996. Vol. 1292, pages 233-240. See figs 2 and 3. Has sequences matching SEQ ID NOs: 18, 19, 21, 43.	1, 2, 4-10 --- 11-20
X --- Y	a-geneseq31-2, R12814, JP 03-099099 (TSUMURA & Co.) 24 April 1991. Alignments only has sequences matching SEQ ID NOs: 2 and 14.	2, 4-10 --- 12, 13, 15-20
X	Swiss-Prot 35, P28592, SUZUKI, K. et al., Agric. Biol. Chem. (1 Dec 1992) Vol 45, pages 629-634, Alignment only. Matches SEQ ID NO: 6.	2
Y	CLARK et al. Protease Inhibitors Suppress In Vitro Growth of Huma Small Lung Cancer. 1993. Peptides. Vol. 14, No. 5, pages 1021-1028, see entire document.	11-20

Form PCT/ISA/210 (continuation of second sheet)(July 1992)*

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US98/03642

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

2. ☐ Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. ☐ Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

Please See Extra Sheet.

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. ☒ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
1-20

4. ☐ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

☐ The additional search fees were accompanied by the applicant's protest.

☐ No protest accompanied the payment of additional search fees.

B. FIELDS SEARCHED
Electronic data bases consulted (Name of data base and where practicable terms used):

Sequence search of SEQ ID NO: 1-25, and 43-48; Medline, CAplus, Scisearch
search terms: propeptide or prohormone, convertase, tumor or virus or viral and treatment

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION WAS LACKING
This ISA found multiple inventions as follows:

This application contains the following inventions or groups of inventions which are not so linked as to form a single inventive concept under PCT Rule 13.1. In order for all inventions to be searched, the appropriate additional search fees must be paid.

Group I, claims 1-10, drawn to various peptides.

Group II, claims 11-20, drawn to the first method of use of peptides of Group I.

Group III, claims 21-26, drawn to the second method of use of peptides of Group I.

Group IV, claims 27-31, drawn to the nucleic acids encoding peptides of claims 1 or 2 and the corresponding expression vectors.

Group V, claims 33-38, drawn to expression vector as per group V linked to a nucleic acid encoding a carrier peptide.

Group VI, claim 32, drawn to a chimeric protein (peptide of claims 1-2 fused to a signal peptide).

Group VII, claim 39, drawn to a chimeric protein (peptide of claims 1-2 fused to a prohormone).

The inventions of Groups I-VII are directed to more than one species of the generic invention. These species are deemed to lack Unity of Invention because they are not so linked as to form a single inventive concept under PCT Rule 13.1. The species are as follows:

(a)SEQ ID Nos: 43-46, 1, 4, 5, 9, 10, 12, 13, 16, 17, 19, 20, 22, 25;
(b)SEQ ID Nos: 2, 6, 14, 15, 18;
(c)SEQ ID Nos: 3,7, 8, 11;
(d)SEQ ID Nos: 47, 48, 21, 24.

The inventions listed as Groups I-VII do not relate to a single inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: The inventions listed as Groups I-VIII do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features. The peptides encompassed by claims 1-3 are drawn to several groups of products which do share a mixture of structural motifs but not a single common structure, i.e., there is no structurally distinctive portion of the structure which is shared by all of the alternatives. Further, the peptides of claim 1 are not the contribution over the prior art because they are suggested by references teaching the structure limitation of the peptides of the claim, i.e., peptides as defined in claims 1-3. For example, the peptide

1 MRFPSIFTAV LFAASSALAA PVNTTTEDET AQIPAEAVIG YSDLEGDFDV
51 AVLPFSNSTN NGLLFINTTI ASIAAKEEGV SLDKRGLYAP SALVLTMGHG
101 NSAATVNPER AVTLNCAPTA SGTHPAALQA CAELRGAGGD FDALTVRGDV 151
ACTKQFDPVV VTVDGVWQGK RVSYERTFAN ECVKNSYGMT VFTF

wherein the sequence in bold is the sequence of SEQ ID No:1, is described in WO 9317086 (DATABASE REGISTRY, RN 156200-11-2). Therefore, the lack of unity is present because the linking technical feature is not a "special technical feature" as defined by PCT Rule 13.2.

The first method of use (claims 11-20) will be examined together with the product of Group I in the scope commensurate with the elected product species (see below). The inventions of Groups III-VIII will be examined, in the scope commensurate with the elected product species, if the appropriate additional examination fees are paid.

The peptides of SEQ ID NOs: 1-25 and 43-48 have been separated into groups (a)-(d) so as to represent groups of peptides appearing to have the closest structural relationships.